

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau



(43) International Publication Date
28 August 2003 (28.08.2003)

PCT

(10) International Publication Number
WO 03/070750 A2

(51) International Patent Classification⁷: C07K

(74) Agent: TERPSTRA, Anita, J.; McDonnell Boehnen Hulbert & Berghoff, Suite 3200, 300 South Wacker Drive, Chicago, IL 60606 (US).

(21) International Application Number: PCT/US03/05043

(22) International Filing Date: 20 February 2003 (20.02.2003)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:

60/358,580	20 February 2002 (20.02.2002)	US
60/363,124	11 March 2002 (11.03.2002)	US
PCT/US02/09187	26 March 2002 (26.03.2002)	US
60/386,782	6 June 2002 (06.06.2002)	US
60/401,104	5 August 2002 (05.08.2002)	US
60/406,784	29 August 2002 (29.08.2002)	US
60/408,378	5 September 2002 (05.09.2002)	US
60/409,293	9 September 2002 (09.09.2002)	US
60/440,129	15 January 2003 (15.01.2003)	US

(81) Designated States (*national*): AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW.

(84) Designated States (*regional*): ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PT, SE, SI, SK, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

(71) Applicant (*for all designated States except US*): SIRNA THERAPEUTICS, INC [US/US]; 2950 Wilderness Place, Boulder, CO 80301 (US).

Declaration under Rule 4.17:

— of inventorship (Rule 4.17(iv)) for US only

(72) Inventors; and

(75) Inventors/Applicants (*for US only*): McSWIGGEN, James [US/US]; 4866 Franklin Drive, Boulder, CO 80301 (US). BEIGELMAN, Leonid [US/US]; 5530 Colt Drive, Longmont, CO 80503 (US). MACEJAK, Dennis [US/US]; 6595 Union Street, Arvada, CO 80004 (US). MORRISSEY, David [US/US]; 4769 Tanglewood Trail, Boulder, CO 80301 (US).

Published:

— without international search report and to be republished upon receipt of that report

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(54) Title: RNA INTERFERENCE MEDIATED INHIBITION OF HEPATITIS C VIRUS (HCV) GENE EXPRESSION USING SHORT INTERFERING NUCLEIC ACID (siNA)

(57) Abstract: The present invention concerns methods and reagents useful in modulating hepatitis C virus (HCV) gene expression in a variety of applications, including use in therapeutic, diagnostic, target validation, and genomic discovery applications. Specifically, the invention relates to small nucleic acid molecules, such as short interfering nucleic acid (siNA), short interfering RNA (siRNA), double-stranded RNA (dsRNA), micro-RNA (miRNA), and short hairpin RNA (shRNA) molecules capable of mediating RNA interference (RNAi) against hepatitis C virus (HCV) gene expression and/or activity. The small nucleic acid molecules are useful in the treatment and diagnosis of HCV infection, liver failure, hepatocellular carcinoma, cirrhosis and any other disease or condition that responds to modulation of HCV expression or activity.

WO 03/070750 A2

**RNA INTERFERENCE MEDIATED INHIBITION OF HEPATITIS C VIRUS
(HCV) GENE EXPRESSION USING SHORT INTERFERING NUCLEIC ACID
(siNA)**

This invention claims the benefit of McSwiggen PCT/US02/09187 filed March 26,
5 2002, McSwiggen USSN 60/401,104 filed August 5, 2002, of Beigelman USSN
60/358,580 filed February 20, 2002, of Beigelman USSN 60/363,124 filed March 11,
2002, of Beigelman USSN 60/386,782 filed June 6, 2002, of Beigelman USSN
60/406,784 filed August 29, 2002, of Beigelman USSN 60/408,378 filed September 5,
2002, of Beigelman USSN 60/409,293 filed September 9, 2002, and of Beigelman USSN
10 60/440,129 filed January 15, 2003. These applications are hereby incorporated by
reference herein in their entireties, including the drawings.

Field Of The Invention

The present invention concerns compounds, compositions, and methods for the
study, diagnosis, and treatment of conditions and diseases that respond to the modulation
15 of hepatitis C virus (HCV) gene expression and/or activity. The present invention also
concerns compounds, compositions, and methods relating to conditions and diseases that
respond to the modulation of expression and/or activity of genes involved in HCV
pathways. Specifically, the invention relates to small nucleic acid molecules, such as
short interfering nucleic acid (siNA), short interfering RNA (siRNA), double-stranded
20 RNA (dsRNA), micro-RNA (miRNA), and short hairpin RNA (shRNA) molecules
capable of mediating RNA interference (RNAi) against hepatitis C virus (HCV) gene
expression.

Background Of The Invention

The following is a discussion of relevant art pertaining to RNAi. The discussion is
25 provided only for understanding of the invention that follows. The summary is not an
admission that any of the work described below is prior art to the claimed invention.

RNA interference refers to the process of sequence-specific post-transcriptional
gene silencing in animals mediated by short interfering RNAs (siRNAs) (Fire *et al.*,
1998, *Nature*, 391, 806). The corresponding process in plants is commonly referred to as

post-transcriptional gene silencing or RNA silencing and is also referred to as quelling in fungi. The process of post-transcriptional gene silencing is thought to be an evolutionarily-conserved cellular defense mechanism used to prevent the expression of foreign genes and is commonly shared by diverse flora and phyla (Fire *et al.*, 1999, *Trends Genet.*, 15, 358). Such protection from foreign gene expression may have evolved in response to the production of double-stranded RNAs (dsRNAs) derived from viral infection or from the random integration of transposon elements into a host genome via a cellular response that specifically destroys homologous single-stranded RNA or viral genomic RNA. The presence of dsRNA in cells triggers the RNAi response though a mechanism that has yet to be fully characterized. This mechanism appears to be different from the interferon response that results from dsRNA-mediated activation of protein kinase PKR and 2',5'-oligoadenylate synthetase resulting in non-specific cleavage of mRNA by ribonuclease L.

The presence of long dsRNAs in cells stimulates the activity of a ribonuclease III enzyme referred to as dicer. Dicer is involved in the processing of the dsRNA into short pieces of dsRNA known as short interfering RNAs (siRNAs) (Berstein *et al.*, 2001, *Nature*, 409, 363). Short interfering RNAs derived from dicer activity are typically about 21 to about 23 nucleotides in length and comprise about 19 base pair duplexes (Elbashir *et al.*, 2001, *Genes Dev.*, 15, 188). Dicer has also been implicated in the excision of 21- and 22-nucleotide small temporal RNAs (stRNAs) from precursor RNA of conserved structure that are implicated in translational control (Hutvagner *et al.*, 2001, *Science*, 293, 834). The RNAi response also features an endonuclease complex, commonly referred to as an RNA-induced silencing complex (RISC), which mediates cleavage of single-stranded RNA having sequence complementary to the antisense strand of the siRNA duplex. Cleavage of the target RNA takes place in the middle of the region complementary to the antisense strand of the siRNA duplex (Elbashir *et al.*, 2001, *Genes Dev.*, 15, 188).

RNAi has been studied in a variety of systems. Fire *et al.*, 1998, *Nature*, 391, 806, were the first to observe RNAi in *C. elegans*. Wianny and Goetz, 1999, *Nature Cell Biol.*, 2, 70, describe RNAi mediated by dsRNA in mouse embryos. Hammond *et al.*, 2000, *Nature*, 404, 293, describe RNAi in *Drosophila* cells transfected with dsRNA. Elbashir *et al.*, 2001, *Nature*, 411, 494, describe RNAi induced by introduction of

duplexes of synthetic 21-nucleotide RNAs in cultured mammalian cells including human embryonic kidney and HeLa cells. Recent work in *Drosophila* embryonic lysates (Elbashir *et al.*, 2001, *EMBO J.*, 20, 6877) has revealed certain requirements for siRNA length, structure, chemical composition, and sequence that are essential to mediate efficient RNAi activity. These studies have shown that 21-nucleotide siRNA duplexes are most active when containing 3'-terminal dinucleotide overhangs. Furthermore, complete substitution of one or both siRNA strands with 2'-deoxy (2'-H) or 2'-O-methyl nucleotides abolishes RNAi activity, whereas substitution of the 3'-terminal siRNA overhang nucleotides with 2'-deoxy nucleotides (2'-H) was shown to be tolerated. Single mismatch sequences in the center of the siRNA duplex were also shown to abolish RNAi activity. In addition, these studies also indicate that the position of the cleavage site in the target RNA is defined by the 5'-end of the siRNA guide sequence rather than the 3'-end of the guide sequence (Elbashir *et al.*, 2001, *EMBO J.*, 20, 6877). Other studies have indicated that a 5'-phosphate on the target-complementary strand of a siRNA duplex is required for siRNA activity and that ATP is utilized to maintain the 5'-phosphate moiety on the siRNA (Nykanen *et al.*, 2001, *Cell*, 107, 309).

Studies have shown that replacing the 3'-terminal nucleotide overhanging segments of a 21-mer siRNA duplex having two -nucleotide 3'-overhangs with deoxyribonucleotides does not have an adverse effect on RNAi activity. Replacing up to four nucleotides on each end of the siRNA with deoxyribonucleotides has been reported to be well tolerated, whereas complete substitution with deoxyribonucleotides results in no RNAi activity (Elbashir *et al.*, 2001, *EMBO J.*, 20, 6877). In addition, Elbashir *et al.*, *supra*, also report that substitution of siRNA with 2'-O-methyl nucleotides completely abolishes RNAi activity. Li *et al.*, International PCT Publication No. WO 00/44914, and Beach *et al.*, International PCT Publication No. WO 01/68836 preliminarily suggest that siRNA may include modifications to either the phosphate-sugar backbone or the nucleoside to include at least one of a nitrogen or sulfur heteroatom, however, neither application postulates to what extent such modifications would be tolerated in siRNA molecules, nor provides any further guidance or examples of such modified siRNA. Kreutzer *et al.*, Canadian Patent Application No. 2,359,180, also describe certain chemical modifications for use in dsRNA constructs in order to counteract activation of double-stranded RNA-dependent protein kinase PKR, specifically 2'-amino or 2'-O-methyl nucleotides, and nucleotides containing a 2'-O or 4'-C methylene bridge.

However, Kreutzer *et al.* similarly fails to provide examples or guidance as to what extent these modifications would be tolerated in siRNA molecules.

Parrish *et al.*, 2000, *Molecular Cell*, 6, 1977-1087, tested certain chemical modifications targeting the unc-22 gene in *C. elegans* using long (>25 nt) siRNA transcripts. The authors describe the introduction of thiophosphate residues into these
5 siRNA transcripts by incorporating thiophosphate nucleotide analogs with T7 and T3 RNA polymerase and observed that RNAs with two phosphorothioate modified bases also had substantial decreases in effectiveness as RNAi. Further, Parrish *et al.* reported that phosphorothioate modification of more than two residues greatly destabilized the
10 RNAs *in vitro* such that interference activities could not be assayed. *Id.* at 1081. The authors also tested certain modifications at the 2'-position of the nucleotide sugar in the long siRNA transcripts and found that substituting deoxynucleotides for ribonucleotides produced a substantial decrease in interference activity, especially in the case of Uridine to Thymidine and/or Cytidine to deoxy-Cytidine substitutions. *Id.* In addition, the
15 authors tested certain base modifications, including substituting, in sense and antisense strands of the siRNA, 4-thiouracil, 5-bromouracil, 5-iodouracil, and 3-(aminoallyl)uracil for uracil, and inosine for guanosine. Whereas 4-thiouracil and 5-bromouracil substitution appeared to be tolerated, Parrish reported that inosine produced a substantial decrease in interference activity when incorporated in either strand. Parrish also reported
20 that incorporation of 5-iodouracil and 3-(aminoallyl)uracil in the antisense strand resulted in a substantial decrease in RNAi activity as well.

The use of longer dsRNA has been described. For example, Beach *et al.*, International PCT Publication No. WO 01/68836, describes specific methods for attenuating gene expression using endogenously-derived dsRNA. Tuschl *et al.*,
25 International PCT Publication No. WO 01/75164, describe a *Drosophila in vitro* RNAi system and the use of specific siRNA molecules for certain functional genomic and certain therapeutic applications; although Tuschl, 2001, *Chem. Biochem.*, 2, 239-245, doubts that RNAi can be used to cure genetic diseases or viral infection due to the danger of activating interferon response. Li *et al.*, International PCT Publication No. WO
30 00/44914, describe the use of specific dsRNAs for attenuating the expression of certain target genes. Zernicka-Goetz *et al.*, International PCT Publication No. WO 01/36646, describe certain methods for inhibiting the expression of particular genes in mammalian

cells using certain dsRNA molecules. Fire *et al.*, International PCT Publication No. WO 99/32619, describe particular methods for introducing certain dsRNA molecules into cells for use in inhibiting gene expression. Plaetinck *et al.*, International PCT Publication No. WO 00/01846, describe certain methods for identifying specific genes responsible for conferring a particular phenotype in a cell using specific dsRNA molecules. Mello *et al.*, International PCT Publication No. WO 01/29058, describe the identification of specific genes involved in dsRNA-mediated RNAi. Deschamps Depaillette *et al.*, International PCT Publication No. WO 99/07409, describe specific compositions consisting of particular dsRNA molecules combined with certain anti-viral agents. Waterhouse *et al.*, International PCT Publication No. 99/53050, describe certain methods for decreasing the phenotypic expression of a nucleic acid in plant cells using certain dsRNAs. Driscoll *et al.*, International PCT Publication No. WO 01/49844, describe specific DNA constructs for use in facilitating gene silencing in targeted organisms.

Others have reported on various RNAi and gene-silencing systems. For example, Parrish *et al.*, 2000, *Molecular Cell*, 6, 1977-1087, describe specific chemically-modified siRNA constructs targeting the unc-22 gene of *C. elegans*. Grossniklaus, International PCT Publication No. WO 01/38551, describes certain methods for regulating polycomb gene expression in plants using certain dsRNAs. Churikov *et al.*, International PCT Publication No. WO 01/42443, describe certain methods for modifying genetic characteristics of an organism using certain dsRNAs. Cogoni *et al.*, International PCT Publication No. WO 01/53475, describe certain methods for isolating a *Neurospora* silencing gene and uses thereof. Reed *et al.*, International PCT Publication No. WO 01/68836, describe certain methods for gene silencing in plants. Honer *et al.*, International PCT Publication No. WO 01/70944, describe certain methods of drug screening using transgenic nematodes as Parkinson's Disease models using certain dsRNAs. Deak *et al.*, International PCT Publication No. WO 01/72774, describe certain *Drosophila*-derived gene products that may be related to RNAi in *Drosophila*. Arndt *et al.*, International PCT Publication No. WO 01/92513 describe certain methods for mediating gene suppression by using factors that enhance RNAi. Tuschl *et al.*, International PCT Publication No. WO 02/44321, describe certain synthetic siRNA constructs. Pachuk *et al.*, International PCT Publication No. WO 00/63364, and Satishchandran *et al.*, International PCT Publication No. WO 01/04313, describe certain

methods and compositions for inhibiting the function of certain polynucleotide sequences using certain dsRNAs. Echeverri *et al.*, International PCT Publication No. WO 02/38805, describe certain *C. elegans* genes identified via RNAi. Kreutzer *et al.*, International PCT Publications Nos. WO 02/055692, WO 02/055693, and EP 1144623 B1 describes certain methods for inhibiting gene expression using RNAi. Graham *et al.*, International PCT Publications Nos. WO 99/49029 and WO 01/70949, and AU 4037501 describe certain vector expressed siRNA molecules. Fire *et al.*, US 6,506,559, describe certain methods for inhibiting gene expression in vitro using certain certain long dsRNA (greater than 25 nucleotide) constructs that mediate RNAi.

10 McCaffrey *et al.*, 2002, *Nature*, 418, 38-39, describes the use of certain siRNA constructs targeting a chimeric HCV NS5B protein/luciferase transcript in mice.

Randall *et al.*, 2003, *PNAS USA*, 100, 235-240, describe certain siRNA constructs targeting HCV RNA in Huh7 hepatoma cell lines.

SUMMARY OF THE INVENTION

15 This invention relates to compounds, compositions, and methods useful for modulating the expression of genes, such as those genes associated with the development or maintenance of HCV infection, liver failure, hepatocellular carcinoma, cirrhosis, and/or other disease states associated with HCV infection, by RNA interference (RNAi), using short interfering nucleic acid (siNA) molecules. This invention also relates to
20 compounds, compositions, and methods useful for modulating the expression and activity of hepatitis C virus (HCV), or genes involved in hepatitis C virus (HCV) gene expression and/or activity by RNA interference (RNAi) using small nucleic acid molecules, such as short interfering nucleic acid (siNA), short interfering RNA (siRNA), double-stranded RNA (dsRNA), micro-RNA (miRNA), and short hairpin RNA (shRNA)
25 molecules. In particular, the instant invention features small nucleic acid molecules, such as short interfering nucleic acid (siNA), short interfering RNA (siRNA), double-stranded RNA (dsRNA), micro-RNA (miRNA), and short hairpin RNA (shRNA) molecules and methods used to modulate the expression of hepatitis C virus (HCV). A siNA of the invention can be unmodified or chemically-modified. A siNA of the instant
30 invention can be chemically synthesized, expressed from a vector or enzymatically synthesized. The instant invention also features various chemically-modified synthetic

short interfering nucleic acid (siNA) molecules capable of modulating hepatitis C virus gene expression or activity in cells by RNA interference (RNAi). The use of chemically-modified siNA improves various properties of native siNA molecules through increased resistance to nuclease degradation *in vivo* and/or through improved cellular uptake.

5 Further, contrary to earlier published studies, siNA having multiple chemical modifications retains its RNAi activity. The siNA molecules of the instant invention provide useful reagents and methods for a variety of therapeutic, diagnostic, target validation, genomic discovery, genetic engineering, and pharmacogenomic applications.

In one embodiment, the invention features one or more siNA molecules and

10 methods that independently or in combination modulate the expression of gene(s) encoding the hepatitis C virus. Specifically, the present invention features siNA molecules that modulate the expression of HCV proteins, for example, proteins encoded by sequences shown as Genbank Accession Nos. in Table I.

In one embodiment, the invention features siNA molecules having RNAi

15 specificity for the HCV minus strand, for example, Genbank Accession No. HPCCK1S1, Hepatitis C virus (strain HCV-1b, clone HCV-K1-S1), complete genome; Genbank Accession No. D50483; 9410 nt.

In one embodiment, the invention features one or more siNA molecules and methods that independently or in combination modulate the expression of genes

20 representing cellular targets for HCV infection, such as cellular receptors, cell surface molecules, cellular enzymes, cellular transcription factors, and/or cytokines, second messengers, and cellular accessory molecules including, but not limited to, interferon regulatory factors (IRFs; e.g., Genbank Accession No. AF082503.1); cellular PKR protein kinase (e.g., Genbank Accession No. XM_002661.7); human eukaryotic

25 initiation factors 2B (eIF2Bgamma; e.g., Genbank Accession No. AF256223, and/or eIF2gamma; e.g., Genbank Accession No. NM_006874.1); human DEAD Box protein (DDX3; e.g., Genbank Accession No. XM_018021.2); and cellular proteins that bind to the poly(U) tract of the HCV 3'-UTR, such as polypyrimidine tract-binding protein (e.g., Genbank Accession Nos. NM_031991.1 and XM_042972.3).

30 Due to the high sequence variability of the HCV genome, selection of siNA molecules for broad therapeutic applications would likely involve the conserved regions

of the HCV genome. In one embodiment, the present invention relates to siNA molecules that target the conserved regions of the HCV genome. Examples of conserved regions of the HCV genome include, but are not limited to, the 5'-Non Coding Region (NCR, also referred to as 5'-untranscribed region, UTR), the 5'-end of the core protein coding region, and the 3'- NCR. HCV genomic RNA contains an internal ribosome entry site (IRES) in the 5'-NCR which mediates translation independently of a 5'-cap structure (Wang *et al.*, 1993, *J. Virol.*, 67, 3338-44). The full-length sequence of the HCV RNA genome is heterologous among clinically isolated subtypes, of which there are at least fifteen (Simmonds, 1995, *Hepatology*, 21, 570-583), however, the 5'-NCR sequence of HCV is highly conserved across all known subtypes, most likely to preserve the shared IRES mechanism (Okamoto *et al.*, 1991, *J. General Virol.*, 72, 2697-2704). Therefore, a siNA molecule can be designed to target all the different isolates of HCV. siNA molecules designed to target conserved regions of various HCV isolates can enable efficient inhibition of HCV replication in diverse patient populations and can ensure the effectiveness of the siNA molecules against HCV quasi species which evolve due to mutations in the non-conserved regions of the HCV genome. Therefore, a single siNA molecule can be targeted against all isolates of HCV by designing the siNA molecule to interact with conserved nucleotide sequences of HCV (such conserved sequences are expected to be present in the RNA of all HCV isolates).

In one embodiment, the invention features one or more siNA molecules and methods that independently or in combination modulate the expression of gene(s) encoding HCV and/or cellular proteins associated with the maintenance or development of HCV infection, liver failure, hepatocellular carcinoma, and cirrhosis, such as genes encoding sequences comprising those sequences referred to by GenBank Accession Nos. shown in Table I, referred to herein generally as HCV. The description below of the various aspects and embodiments of the invention is provided with reference to exemplary hepatitis C virus (HCV) genes, generally referred to herein as HCV. However, such reference is meant to be exemplary only and the various aspects and embodiments of the invention are also directed to other genes that express alternate HCV genes, such as mutant HCV genes, splice variants of HCV genes, and genes encoding different strains of HCV, as well as as cellular targets for HCV, such as those described herein. The various aspects and embodiments are also directed to other genes involved

in HCV pathways, including genes that encode cellular proteins involved in the maintenance and/or development of HCV infection, liver failure, hepatocellular carcinoma, and cirrhosis or other genes that express other proteins associated with HCV infection, such as cellular proteins that are utilized in the HCV life-cycle. Such additional genes can be analyzed for target sites using the methods described herein for HCV. Thus, the inhibition and the effects of such inhibition of the other genes can be performed as described herein. In other words, the term "HCV" as it is defined herein below and recited in the described embodiments, is meant to encompass genes associated with the development or maintenance of HCV infection, such as genes which encode HCV polypeptides, including polypeptides of different strains of HCV, mutant HCV genes, and splice variants of HCV genes, as well as cellular genes involved in HCV pathways of gene expression, replication, and/or HCV activity. Also, the term "HCV" as it is defined herein below and recited in the described embodiments, is meant to encompass HCV viral gene products and cellular gene products involved in HCV infection, such as those described herein. Thus, each of the embodiments described herein with reference to the term "HCV" are applicable to all of the virus, cellular and viral protein, peptide, polypeptide, and/or polynucleotide molecules covered by the term "HCV", as that term is defined herein.

In one embodiment, the invention features a siNA molecule that down-regulates expression of a HCV gene, for example, wherein the HCV gene comprises HCV encoding sequence.

In one embodiment, the invention features a siNA molecule having RNAi activity against HCV RNA, wherein the siNA molecule comprises a sequence complementary to any RNA having HCV or other HCV encoding sequence, such as those sequences having GenBank Accession Nos. shown in Table I. Chemical modifications as shown in Tables III and IV or otherwise described herein can be applied to any siNA construct of the invention.

In one embodiment, the invention features a siNA molecule having RNAi activity against HCV RNA, wherein the siNA molecule comprises a sequence complementary to any RNA having HCV encoding sequence, such as those sequences having HCV GenBank Accession Nos. shown in Table I. Chemical modifications as shown in

Tables III and IV or otherwise described herein can be applied to any siNA construct of the invention.

In another embodiment, the invention features a siNA molecule having RNAi activity against a HCV gene, wherein the siNA molecule comprises nucleotide sequence complementary to nucleotide sequence of a HCV gene, such as those HCV sequences having GenBank Accession Nos. shown in Table I. In another embodiment, a siNA molecule of the invention includes nucleotide sequence that can interact with nucleotide sequence of a HCV gene and thereby mediate silencing of HCV gene expression, for example, wherein the siNA mediates regulation of HCV gene expression by cellular processes that modulate the chromatin structure of the HCV gene and prevent transcription of the HCV gene.

In another embodiment, the invention features a siNA molecule comprising nucleotide sequence, for example, nucleotide sequence in the antisense region of the siNA molecule that is complementary to a nucleotide sequence or portion of sequence of a HCV gene. In another embodiment, the invention features a siNA molecule comprising a region, for example, the antisense region of the siNA construct, complementary to a sequence or portion of sequence comprising a HCV gene sequence.

In one embodiment, the antisense region of HCV siNA constructs can comprise a sequence complementary to sequence having any of SEQ ID NOs. 1-696 or 1393-1413. In one embodiment, the antisense region can also comprise sequence having any of SEQ ID NOs. 697-1392, 1414, 1420, 1428-1434, 1456-1462, 1479, 1483, 1489-1491, 1493, 1497-1498, 1500, 1513-1524, 1551, 1556, 1570-1581, 1618, 1620, 1622, 1624, 1626, or 1627. In another embodiment, the sense region of HCV constructs can comprise sequence having any of SEQ ID NOs. 1-696, 1393-1413, 1417-1419, 1421-1427, 1449-1455, 1477, 1481, 1485, 1487, 1494-1496, 1499, 1501-1512, 1549, 1553, 1558-1569, 1582-1593, 1617, 1619, 1621, 1623, or 1625. The sense region can comprise a sequence of SEQ ID NO. 1606 and the antisense region can comprise a sequence of SEQ ID NO. 1607. The sense region can comprise a sequence of SEQ ID NO. 1608 and the antisense region can comprise a sequence of SEQ ID NO. 1609. The sense region can comprise a sequence of SEQ ID NO. 1610 and the antisense region can comprise a sequence of SEQ ID NO. 1611. The sense region can comprise a sequence of SEQ ID NO. 1612 and the

antisense region can comprise a sequence of SEQ ID NO. 1613. The sense region can comprise a sequence of SEQ ID NO. 1614 and the antisense region can comprise a sequence of SEQ ID NO. 1615. The sense region can comprise a sequence of SEQ ID NO. 1612 and the antisense region can comprise a sequence of SEQ ID NO. 1616.

5 In one embodiment, a siNA molecule of the invention comprises any of SEQ ID NOs. 1-1627. The sequences shown in SEQ ID NOs: 1-1627 are not limiting. A siNA molecule of the invention can comprise any contiguous HCV sequence (e.g., about 19 to about 25, or about 19, 20, 21, 22, 23, 24 or 25 contiguous HCV nucleotides).

10 In yet another embodiment, the invention features a siNA molecule comprising a sequence, for example, the antisense sequence of the siNA construct, complementary to a sequence or portion of sequence comprising sequence represented by GenBank Accession Nos. shown in Table I. Chemical modifications in Tables III and IV and described herein can be applied to any siRNA construct of the invention.

15 In one embodiment of the invention a siNA molecule comprises an antisense strand having about 19 to about 29 nucleotides, wherein the antisense strand is complementary to a RNA sequence encoding a HCV protein, and wherein the siNA further comprises a sense strand having about 19 to about 29 (e.g., about 19, 20, 21, 22, 23, 24, 25, 26, 27, 28 or 29) nucleotides, and wherein the sense strand and the antisense strand are distinct nucleotide sequences with at least about 19 complementary
20 nucleotides.

25 In another embodiment of the invention a siNA molecule of the invention comprises an antisense region having about 19 to about 29 (e.g., about 19, 20, 21, 22, 23, 24, 25, 26, 27, 28 or 29) nucleotides, wherein the antisense region is complementary to a RNA sequence encoding a HCV protein, and wherein the siNA further comprises a sense region having about 19 to about 29 nucleotides, wherein the sense region and the antisense region comprise a linear molecule with at least about 19 complementary nucleotides.

30 In one embodiment of the invention a siNA molecule comprises an antisense strand comprising a nucleotide sequence that is complementary to a nucleotide sequence or a portion thereof encoding a HCV protein. The siNA further comprises a sense strand,

wherein the sense strand comprises a nucleotide sequence of a HCV gene or a portion thereof.

In another embodiment, a siNA molecule comprises an antisense region comprising a nucleotide sequence that is complementary to a nucleotide sequence or a portion thereof encoding a HCV protein. The siNA molecule further comprises a sense region, wherein the sense region comprises a nucleotide sequence of a HCV gene or a portion thereof.

In one embodiment, a siNA molecule of the invention has RNAi activity that modulates expression of RNA encoded by a HCV gene. Because HCV genes can share some degree of sequence homology with each other, siNA molecules can be designed to target a class of HCV genes or alternately specific HCV genes by selecting sequences that are either shared amongst different HCV targets or alternatively that are unique for a specific HCV target. Therefore, in one embodiment, the siNA molecule can be designed to target conserved regions of HCV RNA sequence having homology between several HCV genes so as to target several HCV genes (e.g., different HCV isoforms, splice variants, mutant genes etc.) with one siNA molecule. In another embodiment, the siNA molecule can be designed to target a sequence that is unique to a specific HCV RNA sequence due to the high degree of specificity that the siNA molecule requires to mediate RNAi activity.

In one embodiment, nucleic acid molecules of the invention that act as mediators of the RNA interference gene silencing response are double-stranded nucleic acid molecules. In another embodiment, the siNA molecules of the invention consist of duplexes containing about 19 base pairs between oligonucleotides comprising about 19 to about 25 (e.g., about 19, 20, 21, 22, 23, 24 or 25) nucleotides. In yet another embodiment, siNA molecules of the invention comprise duplexes with overhanging ends of about about 1 to about 3 (e.g., about 1, 2, or 3) nucleotides, for example about 21-nucleotide duplexes with about 19 base pairs and 3'-terminal mononucleotide, dinucleotide, or trinucleotide overhangs.

In one embodiment, the invention features one or more chemically-modified siNA constructs having specificity for HCV expressing nucleic acid molecules, such as RNA encoding a HCV protein. Non-limiting examples of such chemical modifications include

without limitation phosphorothioate internucleotide linkages, 2'-deoxyribonucleotides, 2'-O-methyl ribonucleotides, 2'-deoxy-2'-fluoro ribonucleotides, "universal base" nucleotides, "acyclic" nucleotides, 5-C-methyl nucleotides, and terminal glyceryl and/or inverted deoxy abasic residue incorporation. These chemical modifications, when used
5 in various siNA constructs, are shown to preserve RNAi activity in cells while at the same time, dramatically increasing the serum stability of these compounds. Furthermore, contrary to the data published by Parrish *et al.*, *supra*, applicant demonstrates that multiple (greater than one) phosphorothioate substitutions are well-tolerated and confer substantial increases in serum stability for modified siNA
10 constructs.

In one embodiment, a siNA molecule of the invention comprises modified nucleotides while maintaining the ability to mediate RNAi. The modified nucleotides can be used to improve *in vitro* or *in vivo* characteristics such as stability, activity, and/or bioavailability. For example, a siNA molecule of the invention can comprise modified
15 nucleotides as a percentage of the total number of nucleotides present in the siNA molecule. As such, a siNA molecule of the invention can generally comprise about 5% to about 100% modified nucleotides (e.g., 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95% or 100% modified nucleotides). The actual percentage of modified nucleotides present in a given siNA
20 molecule will depend on the total number of nucleotides present in the siNA. If the siNA molecule is single stranded, the percent modification can be based upon the total number of nucleotides present in the single stranded siNA molecules. Likewise, if the siNA molecule is double stranded, the percent modification can be based upon the total number of nucleotides present in the sense strand, antisense strand, or both the sense and
25 antisense strands.

In one embodiment, the invention features a double-stranded short interfering nucleic acid (siNA) molecule that inhibits replication of a hepatitis C virus (HCV), wherein one of the strands of the double-stranded siNA molecule is an antisense strand which comprises a nucleotide sequence that is complementary to the nucleotide sequence
30 of an HCV RNA or a portion thereof and the other strand is a sense strand which comprises a nucleotide sequence that is complementary to the nucleotide sequence of the

antisense strand. In one embodiment, the HCV RNA comprises HCV minus strand RNA. In another embodiment, the HCV RNA comprises HCV plus strand RNA.

In one embodiment, the invention features a double-stranded short interfering nucleic acid (siNA) molecule that inhibits replication of a hepatitis C virus (HCV), wherein one of the strands of the double-stranded siNA molecule is an antisense strand which comprises a nucleotide sequence that is complementary to the nucleotide sequence of an HCV RNA or a portion thereof, and the other strand is a sense strand which comprises a nucleotide sequence that is complementary to the nucleotide sequence of the antisense strand, wherein a majority of the pyrimidine nucleotides present in the double-stranded siNA molecule comprises a sugar modification. In one embodiment, all of the pyrimidine nucleotides present in the double-stranded siNA molecule comprise a sugar modification. In one embodiment, each strand of the double-stranded siNA molecule comprises about 19 to about 29 nucleotides and each strand comprises at least about 19 nucleotides that are complementary to the nucleotides of the other strand. In another embodiment, the double-stranded siNA molecule is assembled from two oligonucleotide fragments, wherein one fragment comprises nucleotide sequence of the antisense strand of the siNA molecule and the second fragment comprises nucleotide sequence of the sense strand of the siNA molecule. In yet another embodiment, the sense strand of the double-stranded siNA molecule is connected to the antisense strand via a linker molecule, such as a polynucleotide linker or a non-nucleotide linker. In another embodiment, any pyrimidine nucleotides (i.e., one or more or all) present in the sense strand of the double-stranded siNA molecule are 2'-deoxy-2'-fluoro pyrimidine nucleotides and any purine nucleotides (i.e., one or more or all) present in the sense region are 2'-deoxy purine nucleotides. In yet another embodiment, the sense strand of the double-stranded siNA molecule comprises a 3'-end and a 5'-end, wherein a terminal cap moiety (e.g., an inverted deoxy abasic moiety) is present at the 5'-end, the 3'-end, or both of the 5' and 3' ends of the sense strand. In another embodiment, the antisense strand of the double-stranded siNA molecule comprises one or more 2'-deoxy-2'-fluoro pyrimidine nucleotides and one or more 2'-O-methyl purine nucleotides. In yet another embodiment, any pyrimidine nucleotides present in the antisense strand of the double-stranded siNA molecule are 2'-deoxy-2'-fluoro pyrimidine nucleotides and any purine nucleotides present in the antisense strand are 2'-O-methyl purine nucleotides. In another embodiment, the antisense strand of the double-stranded siNA molecule comprises a

phosphorothioate internucleotide linkage at the 3' end of the antisense strand. In yet another embodiment, the antisense strand comprises a glyceryl modification at the 3' end of the antisense strand. In still another embodiment, the 5'-end of the antisense strand optionally includes a phosphate group.

5 In one embodiment, the invention features a double-stranded short interfering nucleic acid (siNA) molecule that inhibits replication of a hepatitis C virus (HCV), wherein one of the strands of the double-stranded siNA molecule is an antisense strand which comprises a nucleotide sequence that is complementary to the nucleotide sequence of an HCV RNA or a portion thereof and the other strand is a sense strand which
10 comprises a nucleotide sequence that is complementary to the nucleotide sequence of the antisense strand, wherein a majority of the pyrimidine nucleotides present in the double-stranded siNA molecule comprises a sugar modification, and wherein each of the two strands of said siNA molecule comprises 21 nucleotides. In one embodiment, 21 nucleotides of the antisense strand are base-paired to the nucleotide sequence of the HCV
15 RNA or a portion thereof. In another embodiment, about 19 nucleotides of the antisense strand are base-paired to the nucleotide sequence of the HCV RNA or a portion thereof. In one embodiment, each strand of the siNA molecule is base-paired to the complementary nucleotides of the other strand of the siNA molecule. In another embodiment, about 19 nucleotides of each strand of the siNA molecule are base-paired
20 to the complementary nucleotides of the other strand of the siNA molecule and at least two 3' terminal nucleotides of each strand of the siNA molecule are not base-paired to the nucleotides of the other strand of the siNA molecule. In one embodiment, each of the two 3' terminal nucleotides of each strand of the siNA molecule that are not base-paired are 2'-deoxy-pyrimidines, such as 2'-deoxy-thymidine.

25 In one embodiment, the invention features a double-stranded short interfering nucleic acid (siNA) molecule that inhibits replication of a hepatitis C virus (HCV), wherein one of the strands of the double-stranded siNA molecule is an antisense strand which comprises a nucleotide sequence that is complementary to the nucleotide sequence of an HCV RNA or a portion thereof and the other strand is a sense strand which
30 comprises a nucleotide sequence that is complementary to the nucleotide sequence of the antisense strand, wherein a majority of the pyrimidine nucleotides present in the double-stranded siNA molecule comprises a sugar modification and wherein the nucleotide

sequence of the antisense strand or a portion thereof is complementary to a nucleotide sequence of the 5'-untranslated region of an HCV RNA or a portion thereof.

In another embodiment, the invention features a double-stranded short interfering nucleic acid (siNA) molecule that inhibits replication of a hepatitis C virus (HCV),
5 wherein one of the strands of the double-stranded siNA molecule is an antisense strand which comprises a nucleotide sequence that is complementary to the nucleotide sequence of an HCV RNA or a portion thereof, and the other strand is a sense strand which comprises a nucleotide sequence that is complementary to the nucleotide sequence of the antisense strand, wherein a majority of the pyrimidine nucleotides present in the double-
10 stranded siNA molecule comprises a sugar modification and wherein the nucleotide sequence of the antisense strand or a portion thereof is complementary to a nucleotide sequence of an HCV RNA that is present in the RNA of all HCV.

In one embodiment, the invention features a double-stranded short interfering nucleic acid (siNA) molecule that inhibits replication of a hepatitis C virus (HCV),
15 wherein one of the strands of the double-stranded siNA molecule is an antisense strand which comprises nucleotide sequence that is complementary to the nucleotide sequence of an RNA encoding an HCV protein or a fragment thereof and the other strand is a sense strand which comprises a nucleotide sequence that is complementary to the nucleotide sequence of the antisense strand. In one embodiment, a majority of the
20 pyrimidine nucleotides present in the double-stranded siNA molecule comprises a sugar modification.

In one embodiment, the invention features a pharmaceutical composition comprising a siNA molecule of the invention in an acceptable carrier or diluent.

In one embodiment, the invention features a medicament comprising an siNA
25 molecule of the invention.

In one embodiment, the invention features an active ingredient comprising an siNA molecule of the invention.

In one embodiment, the nucleotide sequence of the antisense strand or a portion thereof of a siNA molecule of the invention is complementary to the nucleotide sequence
30 of an HCV RNA or a portion thereof that is present in the RNA of all HCV isolates.

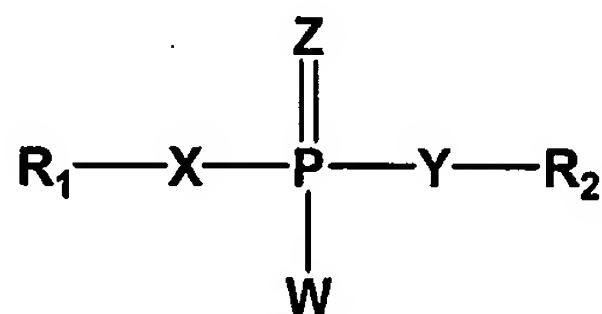
In one embodiment, the invention features the use of a double-stranded short interfering nucleic acid (siNA) molecule that inhibits replication of a hepatitis C virus (HCV), wherein one of the strands of said double-stranded siNA molecule is an antisense strand which comprises a nucleotide sequence that is complementary to the nucleotide sequence of an HCV RNA or a portion thereof and the other strand is a sense strand which comprises a nucleotide sequence that is complementary to the nucleotide sequence of the antisense strand, wherein a majority of the pyrimidine nucleotides present in said double-stranded siNA molecule comprises a sugar modification.

In a non-limiting example, the introduction of chemically-modified nucleotides into nucleic acid molecules provides a powerful tool in overcoming potential limitations of *in vivo* stability and bioavailability inherent to native RNA molecules that are delivered exogenously. For example, the use of chemically-modified nucleic acid molecules can enable a lower dose of a particular nucleic acid molecule for a given therapeutic effect since chemically-modified nucleic acid molecules tend to have a longer half-life in serum. Furthermore, certain chemical modifications can improve the bioavailability of nucleic acid molecules by targeting particular cells or tissues and/or improving cellular uptake of the nucleic acid molecule. Therefore, even if the activity of a chemically-modified nucleic acid molecule is reduced as compared to a native nucleic acid molecule, for example, when compared to an all-RNA nucleic acid molecule, the overall activity of the modified nucleic acid molecule can be greater than that of the native molecule due to improved stability and/or delivery of the molecule. Unlike native unmodified siNA, chemically-modified siNA can also minimize the possibility of activating interferon activity in humans.

The antisense region of a siNA molecule of the invention can comprise a phosphorothioate internucleotide linkage at the 3'-end of said antisense region. The antisense region can comprise about one to about five phosphorothioate internucleotide linkages at the 5'-end of said antisense region. The 3'-terminal nucleotide overhangs of a siNA molecule of the invention can comprise ribonucleotides or deoxyribonucleotides that are chemically-modified at a nucleic acid sugar, base, or backbone. The 3'-terminal nucleotide overhangs can comprise one or more universal base ribonucleotides. The 3'-terminal nucleotide overhangs can comprise one or more acyclic nucleotides.

One embodiment of the invention provides an expression vector comprising a nucleic acid sequence encoding at least one siNA molecule of the invention in a manner that allows expression of the nucleic acid molecule. Another embodiment of the invention provides a mammalian cell comprising such an expression vector. The mammalian cell can be a human cell. The siNA molecule of the expression vector can comprise a sense region and an antisense region. The antisense region can comprise sequence complementary to a RNA or DNA sequence encoding HCV and the sense region can comprise sequence complementary to the antisense region. The siNA molecule can comprise two distinct strands having complementary sense and antisense regions. The siNA molecule can comprise a single strand having complementary sense and antisense regions.

In one embodiment, the invention features a chemically-modified short interfering nucleic acid (siNA) molecule capable of mediating RNA interference (RNAi) against a HCV inside a cell or reconstituted *in vitro* system, wherein the chemical modification comprises one or more (*e.g.*, about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more) nucleotides comprising a backbone modified internucleotide linkage having Formula I:

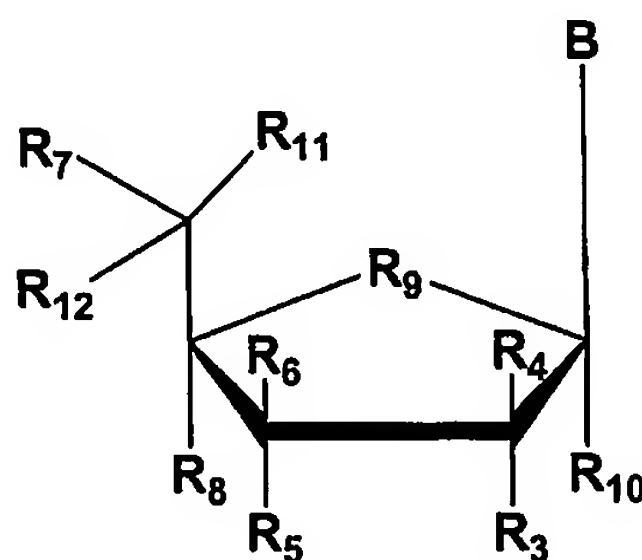


wherein each R1 and R2 is independently any nucleotide, non-nucleotide, or polynucleotide which can be naturally-occurring or chemically-modified, each X and Y is independently O, S, N, alkyl, or substituted alkyl, each Z and W is independently O, S, N, alkyl, substituted alkyl, O-alkyl, S-alkyl, alkaryl, or aralkyl, and wherein W, X, Y, and Z are optionally not all O.

The chemically-modified internucleotide linkages having Formula I, for example, wherein any Z, W, X, and/or Y independently comprises a sulphur atom, can be present in one or both oligonucleotide strands of the siNA duplex, for example, in the sense strand, the antisense strand, or both strands. The siNA molecules of the invention can comprise one or more (*e.g.*, about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more) chemically-modified internucleotide linkages having Formula I at the 3'-end, the 5'-end, or both of

the 3' and 5'-ends of the sense strand, the antisense strand, or both strands. For example, an exemplary siNA molecule of the invention can comprise about 1 to about 5 or more (e.g., about 1, 2, 3, 4, 5, or more) chemically-modified internucleotide linkages having Formula I at the 5'-end of the sense strand, the antisense strand, or both strands. In another non-limiting example, an exemplary siNA molecule of the invention can comprise one or more (e.g., about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more) pyrimidine nucleotides with chemically-modified internucleotide linkages having Formula I in the sense strand, the antisense strand, or both strands. In yet another non-limiting example, an exemplary siNA molecule of the invention can comprise one or more (e.g., about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more) purine nucleotides with chemically-modified internucleotide linkages having Formula I in the sense strand, the antisense strand, or both strands. In another embodiment, a siNA molecule of the invention having internucleotide linkage(s) of Formula I also comprises a chemically-modified nucleotide or non-nucleotide having any of Formulae I-VII.

In one embodiment, the invention features a chemically-modified short interfering nucleic acid (siNA) molecule capable of mediating RNA interference (RNAi) against a HCV inside a cell or reconstituted *in vitro* system, wherein the chemical modification comprises one or more (e.g., about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more) nucleotides or non-nucleotides having Formula II:



20

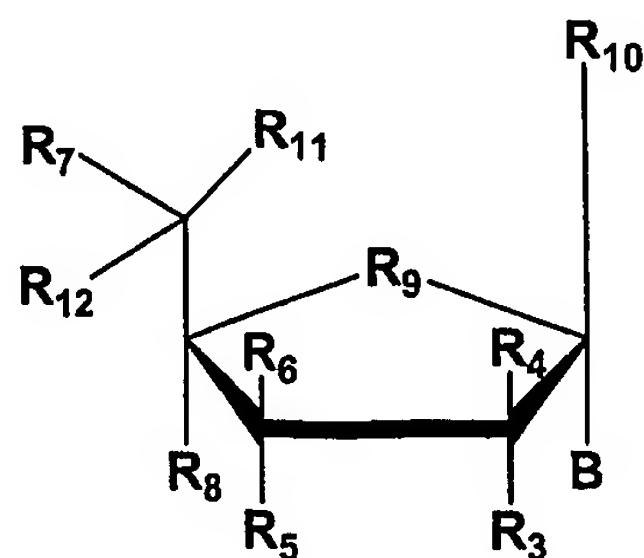
wherein each R3, R4, R5, R6, R7, R8, R10, R11 and R12 is independently H, OH, alkyl, substituted alkyl, alkaryl or aralkyl, F, Cl, Br, CN, CF3, OCF3, OCN, O-alkyl, S-alkyl, N-alkyl, O-alkenyl, S-alkenyl, N-alkenyl, SO-alkyl, alkyl-OSH, alkyl-OH, O-alkyl-OH, O-alkyl-SH, S-alkyl-OH, S-alkyl-SH, alkyl-S-alkyl, alkyl-O-alkyl, ONO2, NO2, N3, NH2, aminoalkyl, aminoacid, aminoacyl, ONH2, O-aminoalkyl, O-aminoacid, O-aminoacyl, heterocycloalkyl, heterocycloalkaryl, aminoalkylamino, polyalkylamino,

25

substituted silyl, or group having Formula I; R₉ is O, S, CH₂, S=O, CHF, or CF₂, and B is a nucleosidic base such as adenine, guanine, uracil, cytosine, thymine, 2-aminoadenosine, 5-methylcytosine, 2,6-diaminopurine, or any other non-naturally occurring base that can be complementary or non-complementary to target RNA or a non-nucleosidic base such as phenyl, naphthyl, 3-nitropyrrole, 5-nitroindole, nebularine, pyridone, pyridinone, or any other non-naturally occurring universal base that can be complementary or non-complementary to target RNA.

The chemically-modified nucleotide or non-nucleotide of Formula II can be present in one or both oligonucleotide strands of the siNA duplex, for example in the sense strand, the antisense strand, or both strands. The siNA molecules of the invention can comprise one or more chemically-modified nucleotide or non-nucleotide of Formula II at the 3'-end, the 5'-end, or both of the 3' and 5'-ends of the sense strand, the antisense strand, or both strands. For example, an exemplary siNA molecule of the invention can comprise about 1 to about 5 or more (*e.g.*, about 1, 2, 3, 4, 5, or more) chemically-modified nucleotides or non-nucleotides of Formula II at the 5'-end of the sense strand, the antisense strand, or both strands. In another non-limiting example, an exemplary siNA molecule of the invention can comprise about 1 to about 5 or more (*e.g.*, about 1, 2, 3, 4, 5, or more) chemically-modified nucleotides or non-nucleotides of Formula II at the 3'-end of the sense strand, the antisense strand, or both strands.

In one embodiment, the invention features a chemically-modified short interfering nucleic acid (siNA) molecule capable of mediating RNA interference (RNAi) against a HCV inside a cell or reconstituted *in vitro* system, wherein the chemical modification comprises one or more (*e.g.*, about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more) nucleotides or non-nucleotides having Formula III:

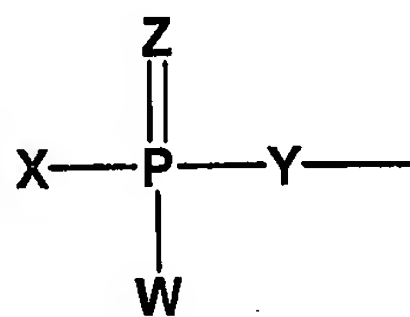


wherein each R3, R4, R5, R6, R7, R8, R10, R11 and R12 is independently H, OH, alkyl, substituted alkyl, alkaryl or aralkyl, F, Cl, Br, CN, CF3, OCF3, OCN, O-alkyl, S-alkyl, N-alkyl, O-alkenyl, S-alkenyl, N-alkenyl, SO-alkyl, alkyl-OSH, alkyl-OH, O-alkyl-OH, O-alkyl-SH, S-alkyl-OH, S-alkyl-SH, alkyl-S-alkyl, alkyl-O-alkyl, ONO2, NO2, N3, NH2, aminoalkyl, aminoacid, aminoacyl, ONH2, O-aminoalkyl, O-aminoacid, O-aminoacyl, heterocycloalkyl, heterocycloalkaryl, aminoalkylamino, polyalkylamino, substituted silyl, or group having Formula I; R9 is O, S, CH2, S=O, CHF, or CF2, and B is a nucleosidic base such as adenine, guanine, uracil, cytosine, thymine, 2-aminoadenosine, 5-methylcytosine, 2,6-diaminopurine, or any other non-naturally occurring base that can be employed to be complementary or non-complementary to target RNA or a non-nucleosidic base such as phenyl, naphthyl, 3-nitropyrrole, 5-nitroindole, nebularine, pyridone, pyridinone, or any other non-naturally occurring universal base that can be complementary or non-complementary to target RNA.

The chemically-modified nucleotide or non-nucleotide of Formula III can be present in one or both oligonucleotide strands of the siNA duplex, for example, in the sense strand, the antisense strand, or both strands. The siNA molecules of the invention can comprise one or more chemically-modified nucleotide or non-nucleotide of Formula III at the 3'-end, the 5'-end, or both of the 3' and 5'-ends of the sense strand, the antisense strand, or both strands. For example, an exemplary siNA molecule of the invention can comprise about 1 to about 5 or more (*e.g.*, about 1, 2, 3, 4, 5, or more) chemically-modified nucleotide(s) or non-nucleotide(s) of Formula III at the 5'-end of the sense strand, the antisense strand, or both strands. In another non-limiting example, an exemplary siNA molecule of the invention can comprise about 1 to about 5 or more (*e.g.*, about 1, 2, 3, 4, 5, or more) chemically-modified nucleotide or non-nucleotide of Formula III at the 3'-end of the sense strand, the antisense strand, or both strands.

In another embodiment, a siNA molecule of the invention comprises a nucleotide having Formula II or III, wherein the nucleotide having Formula II or III is in an inverted configuration. For example, the nucleotide having Formula II or III is connected to the siNA construct in a 3'-3', 3'-2', 2'-3', or 5'-5' configuration, such as at the 3'-end, the 5'-end, or both of the 3' and 5'-ends of one or both siNA strands.

In one embodiment, the invention features a chemically-modified short interfering nucleic acid (siNA) molecule capable of mediating RNA interference (RNAi) against a HCV inside a cell or reconstituted *in vitro* system, wherein the chemical modification comprises a 5'-terminal phosphate group having Formula IV:



wherein each X and Y is independently O, S, N, alkyl, substituted alkyl, or alkylhalo; wherein each Z and W is independently O, S, N, alkyl, substituted alkyl, O-alkyl, S-alkyl, alkaryl, aralkyl, or alkylhalo; and wherein W, X, Y and Z are not all O.

In one embodiment, the invention features a siNA molecule having a 5'-terminal phosphate group having Formula IV on the target-complementary strand, for example, a strand complementary to a target RNA, wherein the siNA molecule comprises an all RNA siNA molecule. In another embodiment, the invention features a siNA molecule having a 5'-terminal phosphate group having Formula IV on the target-complementary strand wherein the siNA molecule also comprises about 1 to about 3 (e.g., about 1, 2, or 3) nucleotide 3'-terminal nucleotide overhangs having about 1 to about 4 (e.g., about 1, 2, 3, or 4) deoxyribonucleotides on the 3'-end of one or both strands. In another embodiment, a 5'-terminal phosphate group having Formula IV is present on the target-complementary strand of a siNA molecule of the invention, for example a siNA molecule having chemical modifications having any of Formulae I-VII.

In one embodiment, the invention features a chemically-modified short interfering nucleic acid (siNA) molecule capable of mediating RNA interference (RNAi) against a HCV inside a cell or reconstituted *in vitro* system, wherein the chemical modification comprises one or more phosphorothioate internucleotide linkages. For example, in a non-limiting example, the invention features a chemically-modified short interfering nucleic acid (siNA) having about 1, 2, 3, 4, 5, 6, 7, 8 or more phosphorothioate internucleotide linkages in one siNA strand. In yet another embodiment, the invention features a chemically-modified short interfering nucleic acid (siNA) individually having about 1, 2, 3, 4, 5, 6, 7, 8 or more phosphorothioate internucleotide linkages in both siNA

strands. The phosphorothioate internucleotide linkages can be present in one or both oligonucleotide strands of the siNA duplex, for example in the sense strand, the antisense strand, or both strands. The siNA molecules of the invention can comprise one or more phosphorothioate internucleotide linkages at the 3'-end, the 5'-end, or both of the 3'- and 5'-ends of the sense strand, the antisense strand, or both strands. For example, an exemplary siNA molecule of the invention can comprise about 1 to about 5 or more (*e.g.*, about 1, 2, 3, 4, 5, or more) consecutive phosphorothioate internucleotide linkages at the 5'-end of the sense strand, the antisense strand, or both strands. In another non-limiting example, an exemplary siNA molecule of the invention can comprise one or more (*e.g.*, about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more) pyrimidine phosphorothioate internucleotide linkages in the sense strand, the antisense strand, or both strands. In yet another non-limiting example, an exemplary siNA molecule of the invention can comprise one or more (*e.g.*, about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more) purine phosphorothioate internucleotide linkages in the sense strand, the antisense strand, or both strands.

15 In one embodiment, the invention features a siNA molecule, wherein the sense strand comprises one or more, for example, about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more phosphorothioate internucleotide linkages, and/or one or more (*e.g.*, about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) 2'-deoxy, 2'-O-methyl, 2'-deoxy-2'-fluoro, and/or one or more (*e.g.*, about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) universal base modified nucleotides, and 20 optionally a terminal cap molecule at the 3'-end, the 5'-end, or both of the 3'- and 5'-ends of the sense strand; and wherein the antisense strand comprises about 1 to about 10 or more, specifically about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more phosphorothioate internucleotide linkages, and/or one or more (*e.g.*, about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) 2'-deoxy, 2'-O-methyl, 2'-deoxy-2'-fluoro, and/or one or more (*e.g.*, about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) universal base modified nucleotides, and optionally a 25 terminal cap molecule at the 3'-end, the 5'-end, or both of the 3'- and 5'-ends of the antisense strand. In another embodiment, one or more, for example about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more, pyrimidine nucleotides of the sense and/or antisense siNA strand are chemically-modified with 2'-deoxy, 2'-O-methyl and/or 2'-deoxy-2'-fluoro nucleotides, 30 with or without one or more, for example about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more, phosphorothioate internucleotide linkages and/or a terminal cap molecule at the 3'-end, the 5'-end, or both of the 3'- and 5'-ends, being present in the same or different strand.

In another embodiment, the invention features a siNA molecule, wherein the sense strand comprises about 1 to about 5, specifically about 1, 2, 3, 4, or 5 phosphorothioate internucleotide linkages, and/or one or more (*e.g.*, about 1, 2, 3, 4, 5, or more) 2'-deoxy, 2'-O-methyl, 2'-deoxy-2'-fluoro, and/or one or more (*e.g.*, about 1, 2, 3, 4, 5, or more) universal base modified nucleotides, and optionally a terminal cap molecule at the 3'-end, the 5'-end, or both of the 3'- and 5'-ends of the sense strand; and wherein the antisense strand comprises about 1 to about 5 or more, specifically about 1, 2, 3, 4, 5, or more phosphorothioate internucleotide linkages, and/or one or more (*e.g.*, about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) 2'-deoxy, 2'-O-methyl, 2'-deoxy-2'-fluoro, and/or one or more (*e.g.*, about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) universal base modified nucleotides, and optionally a terminal cap molecule at the 3'-end, the 5'-end, or both of the 3'- and 5'-ends of the antisense strand. In another embodiment, one or more, for example about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more, pyrimidine nucleotides of the sense and/or antisense siNA strand are chemically-modified with 2'-deoxy, 2'-O-methyl and/or 2'-deoxy-2'-fluoro nucleotides, with or without about 1 to about 5 or more, for example about 1, 2, 3, 4, 5, or more phosphorothioate internucleotide linkages and/or a terminal cap molecule at the 3'-end, the 5'-end, or both of the 3'- and 5'-ends, being present in the same or different strand.

In one embodiment, the invention features a siNA molecule, wherein the antisense strand comprises one or more, for example, about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more phosphorothioate internucleotide linkages, and/or about one or more (*e.g.*, about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) 2'-deoxy, 2'-O-methyl, 2'-deoxy-2'-fluoro, and/or one or more (*e.g.*, about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) universal base modified nucleotides, and optionally a terminal cap molecule at the 3'-end, the 5'-end, or both of the 3'- and 5'-ends of the sense strand; and wherein the antisense strand comprises about 1 to about 10 or more, specifically about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more phosphorothioate internucleotide linkages, and/or one or more (*e.g.*, about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) 2'-deoxy, 2'-O-methyl, 2'-deoxy-2'-fluoro, and/or one or more (*e.g.*, about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) universal base modified nucleotides, and optionally a terminal cap molecule at the 3'-end, the 5'-end, or both of the 3'- and 5'-ends of the antisense strand. In another embodiment, one or more, for example about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more pyrimidine nucleotides of the sense and/or antisense siNA strand are chemically-modified with 2'-deoxy, 2'-O-methyl and/or 2'-deoxy-2'-fluoro nucleotides,

with or without one or more, for example about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more phosphorothioate internucleotide linkages and/or a terminal cap molecule at the 3'-end, the 5'-end, or both of the 3' and 5'-ends, being present in the same or different strand.

In another embodiment, the invention features a siNA molecule, wherein the antisense strand comprises about 1 to about 5 or more, specifically about 1, 2, 3, 4, 5 or more phosphorothioate internucleotide linkages, and/or one or more (*e.g.*, about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) 2'-deoxy, 2'-O-methyl, 2'-deoxy-2'-fluoro, and/or one or more (*e.g.*, about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) universal base modified nucleotides, and optionally a terminal cap molecule at the 3'-end, the 5'-end, or both of the 3'- and 5'-ends of the sense strand; and wherein the antisense strand comprises about 1 to about 5 or more, specifically about 1, 2, 3, 4, 5 or more phosphorothioate internucleotide linkages, and/or one or more (*e.g.*, about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) 2'-deoxy, 2'-O-methyl, 2'-deoxy-2'-fluoro, and/or one or more (*e.g.*, about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) universal base modified nucleotides, and optionally a terminal cap molecule at the 3'-end, the 5'-end, or both of the 3'- and 5'-ends of the antisense strand. In another embodiment, one or more, for example about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more pyrimidine nucleotides of the sense and/or antisense siNA strand are chemically-modified with 2'-deoxy, 2'-O-methyl and/or 2'-deoxy-2'-fluoro nucleotides, with or without about 1 to about 5, for example about 1, 2, 3, 4, 5 or more phosphorothioate internucleotide linkages and/or a terminal cap molecule at the 3'-end, the 5'-end, or both of the 3'- and 5'-ends, being present in the same or different strand.

In one embodiment, the invention features a chemically-modified short interfering nucleic acid (siNA) molecule having about 1 to about 5, specifically about 1, 2, 3, 4, 5 or more phosphorothioate internucleotide linkages in each strand of the siNA molecule.

In another embodiment, the invention features a siNA molecule comprising 2'-5' internucleotide linkages. The 2'-5' internucleotide linkage(s) can be at the 3'-end, the 5'-end, or both of the 3'- and 5'-ends of one or both siNA sequence strands. In addition, the 2'-5' internucleotide linkage(s) can be present at various other positions within one or both siNA sequence strands, for example, about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more including every internucleotide linkage of a pyrimidine nucleotide in one or both strands of the siNA molecule can comprise a 2'-5' internucleotide linkage, or about 1, 2, 3, 4, 5,

6, 7, 8, 9, 10, or more including every internucleotide linkage of a purine nucleotide in one or both strands of the siNA molecule can comprise a 2'-5' internucleotide linkage.

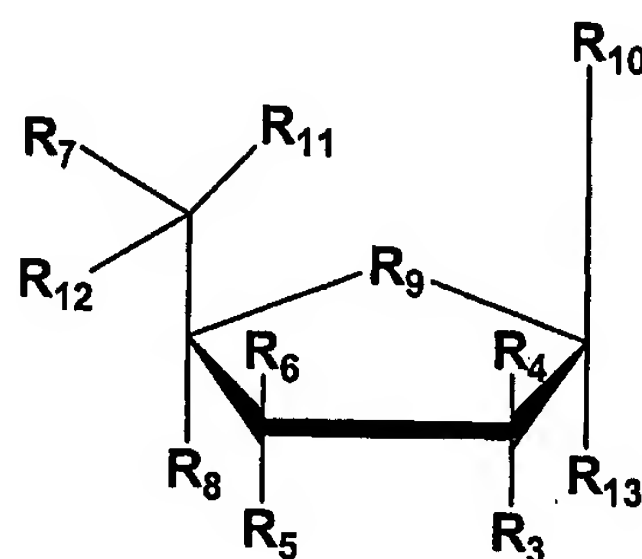
In another embodiment, a chemically-modified siNA molecule of the invention comprises a duplex having two strands, one or both of which can be chemically-
5 modified, wherein each strand is about 18 to about 27 (*e.g.*, about 18, 19, 20, 21, 22, 23, 24, 25, 26, or 27) nucleotides in length, wherein the duplex has about 18 to about 23 (*e.g.*, about 18, 19, 20, 21, 22, or 23) base pairs, and wherein the chemical modification comprises a structure having any of Formulae I-VII. For example, an exemplary chemically-modified siNA molecule of the invention comprises a duplex having two
10 strands, one or both of which can be chemically-modified with a chemical modification having any of Formulae I-VII or any combination thereof, wherein each strand consists of about 21 nucleotides, each having a 2-nucleotide 3'-terminal nucleotide overhang, and wherein the duplex has about 19 base pairs. In another embodiment, a siNA molecule of the invention comprises a single stranded hairpin structure, wherein the siNA is about 36
15 to about 70 (*e.g.*, about 36, 40, 45, 50, 55, 60, 65, or 70) nucleotides in length having about 18 to about 23 (*e.g.*, about 18, 19, 20, 21, 22, or 23) base pairs, and wherein the siNA can include a chemical modification comprising a structure having any of Formulae I-VII or any combination thereof. For example, an exemplary chemically-modified siNA molecule of the invention comprises a linear oligonucleotide having
20 about 42 to about 50 (*e.g.*, about 42, 43, 44, 45, 46, 47, 48, 49, or 50) nucleotides that is chemically-modified with a chemical modification having any of Formulae I-VII or any combination thereof, wherein the linear oligonucleotide forms a hairpin structure having about 19 base pairs and a 2-nucleotide 3'-terminal nucleotide overhang. In another embodiment, a linear hairpin siNA molecule of the invention contains a stem loop motif,
25 wherein the loop portion of the siNA molecule is biodegradable. For example, a linear hairpin siNA molecule of the invention is designed such that degradation of the loop portion of the siNA molecule *in vivo* can generate a double-stranded siNA molecule with 3'-terminal overhangs, such as 3'-terminal nucleotide overhangs comprising about 2 nucleotides.

30 In another embodiment, a siNA molecule of the invention comprises a circular nucleic acid molecule, wherein the siNA is about 38 to about 70 (*e.g.*, about 38, 40, 45, 50, 55, 60, 65, or 70) nucleotides in length having about 18 to about 23 (*e.g.*, about 18,

19, 20, 21, 22, or 23) base pairs, and wherein the siNA can include a chemical modification, which comprises a structure having any of Formulae I-VII or any combination thereof. For example, an exemplary chemically-modified siNA molecule of the invention comprises a circular oligonucleotide having about 42 to about 50 (e.g.,
 5 about 42, 43, 44, 45, 46, 47, 48, 49, or 50) nucleotides that is chemically-modified with a chemical modification having any of Formulae I-VII or any combination thereof, wherein the circular oligonucleotide forms a dumbbell shaped structure having about 19 base pairs and 2 loops.

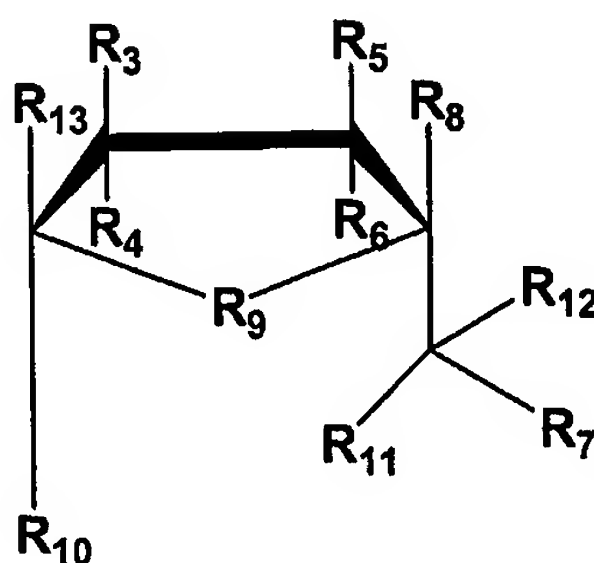
In another embodiment, a circular siNA molecule of the invention contains two
 10 loop motifs, wherein one or both loop portions of the siNA molecule is biodegradable. For example, a circular siNA molecule of the invention is designed such that degradation of the loop portions of the siNA molecule *in vivo* can generate a double-stranded siNA molecule with 3'-terminal overhangs, such as 3'-terminal nucleotide overhangs comprising about 2 nucleotides.

15 In one embodiment, a siNA molecule of the invention comprises at least one (e.g., about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more) abasic moiety, for example a compound having Formula V:



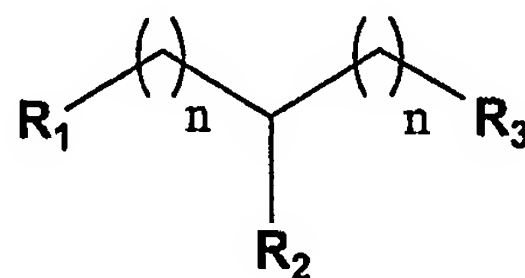
wherein each R3, R4, R5, R6, R7, R8, R10, R11, R12, and R13 is independently H, OH,
 20 alkyl, substituted alkyl, alkaryl or aralkyl, F, Cl, Br, CN, CF3, OCF3, OCN, O-alkyl, S-alkyl, N-alkyl, O-alkenyl, S-alkenyl, N-alkenyl, SO-alkyl, alkyl-OSH, alkyl-OH, O-alkyl-OH, O-alkyl-SH, S-alkyl-OH, S-alkyl-SH, alkyl-S-alkyl, alkyl-O-alkyl, ONO2, NO2, N3, NH2, aminoalkyl, aminoacid, aminoacyl, ONH2, O-aminoalkyl, O-aminoacid, O-aminoacyl, heterocycloalkyl, heterocycloalkaryl, aminoalkylamino, polyalkylamino,
 25 substituted silyl, or group having Formula I; R9 is O, S, CH2, S=O, CHF, or CF2.

In one embodiment, a siNA molecule of the invention comprises at least one (*e.g.*, about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more) inverted abasic moiety, for example a compound having Formula VI:



- 5 wherein each R3, R4, R5, R6, R7, R8, R10, R11, R12, and R13 is independently H, OH, alkyl, substituted alkyl, alkaryl or aralkyl, F, Cl, Br, CN, CF3, OCF3, OCN, O-alkyl, S-alkyl, N-alkyl, O-alkenyl, S-alkenyl, N-alkenyl, SO-alkyl, alkyl-OSH, alkyl-OH, O-alkyl-OH, O-alkyl-SH, S-alkyl-OH, S-alkyl-SH, alkyl-S-alkyl, alkyl-O-alkyl, ONO2, NO2, N3, NH2, aminoalkyl, aminoacid, aminoacyl, ONH2, O-aminoalkyl, O-aminoacid, O-aminoacyl, heterocycloalkyl, heterocycloalkaryl, aminoalkylamino, polyalkylamino, substituted silyl, or group having Formula I; R9 is O, S, CH2, S=O, CHF, or CF2, and either R2, R3, R8 or R13 serve as points of attachment to the siNA molecule of the invention.

- 15 In another embodiment, a siNA molecule of the invention comprises at least one (*e.g.*, about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more) substituted polyalkyl moieties, for example a compound having Formula VII:



- 20 wherein each n is independently an integer from 1 to 12, each R1, R2 and R3 is independently H, OH, alkyl, substituted alkyl, alkaryl or aralkyl, F, Cl, Br, CN, CF3, OCF3, OCN, O-alkyl, S-alkyl, N-alkyl, O-alkenyl, S-alkenyl, N-alkenyl, SO-alkyl, alkyl-OSH, alkyl-OH, O-alkyl-OH, O-alkyl-SH, S-alkyl-OH, S-alkyl-SH, alkyl-S-alkyl, alkyl-O-alkyl, ONO2, NO2, N3, NH2, aminoalkyl, aminoacid, aminoacyl, ONH2, O-aminoalkyl, O-aminoacid, O-aminoacyl, heterocycloalkyl, heterocycloalkaryl,

aminoalkylamino, polyalkylamino, substituted silyl, or a group having Formula I, and R1, R2 or R3 serves as points of attachment to the siNA molecule of the invention.

In another embodiment, the invention features a compound having Formula VII, wherein R1 and R2 are hydroxyl (OH) groups, $n = 1$, and R3 comprises O and is the point of attachment to the 3'-end, the 5'-end, or both of the 3' and 5'-ends of one or both strands of a double-stranded siNA molecule of the invention or to a single-stranded siNA molecule of the invention. This modification is referred to herein as "glyceryl" (for example modification 6 in Figure 10).

In another embodiment, a moiety having any of Formula V, VI or VII of the invention is at the 3'-end, the 5'-end, or both of the 3' and 5'-ends of a siNA molecule of the invention. For example, a moiety having Formula V, VI or VII can be present at the 3'-end, the 5'-end, or both of the 3' and 5'-ends of the antisense strand, the sense strand, or both antisense and sense strands of the siNA molecule. In addition, a moiety having Formula VII can be present at the 3'-end or the 5'-end of a hairpin siNA molecule as described herein.

In another embodiment, a siNA molecule of the invention comprises an abasic residue having Formula V or VI, wherein the abasic residue having Formula VI or VI is connected to the siNA construct in a 3'-3', 3'-2', 2'-3', or 5'-5' configuration, such as at the 3'-end, the 5'-end, or both of the 3' and 5'-ends of one or both siNA strands.

In one embodiment, a siNA molecule of the invention comprises one or more (*e.g.*, about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more) locked nucleic acid (LNA) nucleotides, for example at the 5'-end, the 3'-end, both of the 5' and 3'-ends, or any combination thereof, of the siNA molecule.

In another embodiment, a siNA molecule of the invention comprises one or more (*e.g.*, about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more) acyclic nucleotides, for example at the 5'-end, the 3'-end, both of the 5' and 3'-ends, or any combination thereof, of the siNA molecule.

In one embodiment, the invention features a chemically-modified short interfering nucleic acid (siNA) molecule of the invention, wherein the chemically-modified siNA comprises a sense region, where any (*e.g.*, one or more or all) pyrimidine nucleotides

present in the sense region are 2'-deoxy-2'-fluoro pyrimidine nucleotides (*e.g.*, wherein all pyrimidine nucleotides are 2'-deoxy-2'-fluoro pyrimidine nucleotides or alternately a plurality of pyrimidine nucleotides are 2'-deoxy-2'-fluoro pyrimidine nucleotides), and where any (*e.g.*, one or more or all) purine nucleotides present in the sense region are 2'-
5 deoxy purine nucleotides (*e.g.*, wherein all purine nucleotides are 2'-deoxy purine nucleotides or alternately a plurality of purine nucleotides are 2'-deoxy purine nucleotides).

In one embodiment, the invention features a chemically-modified short interfering nucleic acid (siNA) molecule of the invention, wherein the chemically-modified siNA
10 comprises a sense region, where any (*e.g.*, one or more or all) pyrimidine nucleotides present in the sense region are 2'-deoxy-2'-fluoro pyrimidine nucleotides (*e.g.*, wherein all pyrimidine nucleotides are 2'-deoxy-2'-fluoro pyrimidine nucleotides or alternately a plurality of pyrimidine nucleotides are 2'-deoxy-2'-fluoro pyrimidine nucleotides), and where any (*e.g.*, one or more or all) purine nucleotides present in the sense region are 2'-
15 deoxy purine nucleotides (*e.g.*, wherein all purine nucleotides are 2'-deoxy purine nucleotides or alternately a plurality of purine nucleotides are 2'-deoxy purine nucleotides), wherein any nucleotides comprising a 3'-terminal nucleotide overhang that are present in said sense region are 2'-deoxy nucleotides.

In one embodiment, the invention features a chemically-modified short interfering
20 nucleic acid (siNA) molecule of the invention, wherein the chemically-modified siNA comprises an antisense region, where any (*e.g.*, one or more or all) pyrimidine nucleotides present in the antisense region are 2'-deoxy-2'-fluoro pyrimidine nucleotides (*e.g.*, wherein all pyrimidine nucleotides are 2'-deoxy-2'-fluoro pyrimidine nucleotides or alternately a plurality of pyrimidine nucleotides are 2'-deoxy-2'-fluoro pyrimidine
25 nucleotides), and wherein any (*e.g.*, one or more or all) purine nucleotides present in the antisense region are 2'-O-methyl purine nucleotides (*e.g.*, wherein all purine nucleotides are 2'-O-methyl purine nucleotides or alternately a plurality of purine nucleotides are 2'-O-methyl purine nucleotides).

In one embodiment, the invention features a chemically-modified short interfering
30 nucleic acid (siNA) molecule of the invention, wherein the chemically-modified siNA comprises an antisense region, where any (*e.g.*, one or more or all) pyrimidine

nucleotides present in the antisense region are 2'-deoxy-2'-fluoro pyrimidine nucleotides (e.g., wherein all pyrimidine nucleotides are 2'-deoxy-2'-fluoro pyrimidine nucleotides or alternately a plurality of pyrimidine nucleotides are 2'-deoxy-2'-fluoro pyrimidine nucleotides), and wherein any (e.g., one or more or all) purine nucleotides present in the
5 antisense region are 2'-O-methyl purine nucleotides (e.g., wherein all purine nucleotides are 2'-O-methyl purine nucleotides or alternately a plurality of purine nucleotides are 2'-O-methyl purine nucleotides), wherein any nucleotides comprising a 3'-terminal nucleotide overhang that are present in said antisense region are 2'-deoxy nucleotides.

In one embodiment, the invention features a chemically-modified short interfering
10 nucleic acid (siNA) molecule of the invention, wherein the chemically-modified siNA comprises an antisense region, where any (e.g., one or more or all) pyrimidine nucleotides present in the antisense region are 2'-deoxy-2'-fluoro pyrimidine nucleotides (e.g., wherein all pyrimidine nucleotides are 2'-deoxy-2'-fluoro pyrimidine nucleotides or alternately a plurality of pyrimidine nucleotides are 2'-deoxy-2'-fluoro pyrimidine
15 nucleotides), and where any (e.g., one or more or all) purine nucleotides present in the antisense region are 2'-deoxy purine nucleotides (e.g., wherein all purine nucleotides are 2'-deoxy purine nucleotides or alternately a plurality of purine nucleotides are 2'-deoxy purine nucleotides).

In one embodiment, the invention features a chemically-modified short interfering
20 nucleic acid (siNA) molecule of the invention capable of mediating RNA interference (RNAi) against a HCV inside a cell or reconstituted *in vitro* system, wherein the chemically-modified siNA comprises a sense region, where one or more pyrimidine nucleotides present in the sense region are 2'-deoxy-2'-fluoro pyrimidine nucleotides (e.g., wherein all pyrimidine nucleotides are 2'-deoxy-2'-fluoro pyrimidine nucleotides or
25 alternately a plurality of pyrimidine nucleotides are 2'-deoxy-2'-fluoro pyrimidine nucleotides), and where one or more purine nucleotides present in the sense region are 2'-deoxy purine nucleotides (e.g., wherein all purine nucleotides are 2'-deoxy purine nucleotides or alternately a plurality of purine nucleotides are 2'-deoxy purine nucleotides), and inverted deoxy abasic modifications that are optionally present at the
30 3'-end, the 5'-end, or both of the 3' and 5'-ends of the sense region, the sense region optionally further comprising a 3'-terminal overhang having about 1 to about 4 (e.g., about 1, 2, 3, or 4) 2'-deoxyribonucleotides; and wherein the chemically-modified short

interfering nucleic acid molecule comprises an antisense region, where one or more pyrimidine nucleotides present in the antisense region are 2'-deoxy-2'-fluoro pyrimidine nucleotides (e.g., wherein all pyrimidine nucleotides are 2'-deoxy-2'-fluoro pyrimidine nucleotides or alternately a plurality of pyrimidine nucleotides are 2'-deoxy-2'-fluoro pyrimidine nucleotides), and wherein one or more purine nucleotides present in the antisense region are 2'-O-methyl purine nucleotides (e.g., wherein all purine nucleotides are 2'-O-methyl purine nucleotides or alternately a plurality of purine nucleotides are 2'-O-methyl purine nucleotides), and a terminal cap modification, such as any modification described herein or shown in **Figure 10**, that is optionally present at the 3'-end, the 5'-end, or both of the 3' and 5'-ends of the antisense sequence, the antisense region optionally further comprising a 3'-terminal nucleotide overhang having about 1 to about 4 (e.g., about 1, 2, 3, or 4) 2'-deoxynucleotides, wherein the overhang nucleotides can further comprise one or more (e.g., 1, 2, 3, or 4) phosphorothioate internucleotide linkages. Non-limiting examples of these chemically-modified siNAs are shown in **Figures 4 and 5 and Tables III and IV** herein.

In one embodiment, the invention features a chemically-modified short interfering nucleic acid (siNA) molecule of the invention capable of mediating RNA interference (RNAi) against a HCV inside a cell or reconstituted *in vitro* system, wherein the siNA comprises a sense region, where one or more pyrimidine nucleotides present in the sense region are 2'-deoxy-2'-fluoro pyrimidine nucleotides (e.g., wherein all pyrimidine nucleotides are 2'-deoxy-2'-fluoro pyrimidine nucleotides or alternately a plurality of pyrimidine nucleotides are 2'-deoxy-2'-fluoro pyrimidine nucleotides), and where one or more purine nucleotides present in the sense region are purine ribonucleotides (e.g., wherein all purine nucleotides are purine ribonucleotides or alternately a plurality of purine nucleotides are purine ribonucleotides), and inverted deoxy abasic modifications that are optionally present at the 3'-end, the 5'-end, or both of the 3' and 5'-ends of the sense region, the sense region optionally further comprising a 3'-terminal overhang having about 1 to about 4 (e.g., about 1, 2, 3, or 4) 2'-deoxyribonucleotides; and wherein the siNA comprises an antisense region, where one or more pyrimidine nucleotides present in the antisense region are 2'-deoxy-2'-fluoro pyrimidine nucleotides (e.g., wherein all pyrimidine nucleotides are 2'-deoxy-2'-fluoro pyrimidine nucleotides or alternately a plurality of pyrimidine nucleotides are 2'-deoxy-2'-fluoro pyrimidine nucleotides), and wherein any purine nucleotides present in the antisense region are 2'-O-

5 methyl purine nucleotides (e.g., wherein all purine nucleotides are 2'-O-methyl purine nucleotides or alternately a plurality of purine nucleotides are 2'-O-methyl purine nucleotides), and a terminal cap modification, such as any modification described herein or shown in **Figure 10**, that is optionally present at the 3'-end, the 5'-end, or both of the 3' and 5'-ends of the antisense sequence, the antisense region optionally further comprising a 3'-terminal nucleotide overhang having about 1 to about 4 (e.g., about 1, 2, 3, or 4) 2'-deoxynucleotides, wherein the overhang nucleotides can further comprise one or more (e.g., 1, 2, 3, or 4) phosphorothioate internucleotide linkages. Non-limiting examples of these chemically-modified siNAs are shown in **Figures 4 and 5 and Tables**
10 **III and IV** herein.

In one embodiment, the invention features a chemically-modified short interfering nucleic acid (siNA) molecule of the invention capable of mediating RNA interference (RNAi) against a HCV inside a cell or reconstituted *in vitro* system, wherein the chemically-modified siNA comprises a sense region, where one or more pyrimidine
15 nucleotides present in the sense region are 2'-deoxy-2'-fluoro pyrimidine nucleotides (e.g., wherein all pyrimidine nucleotides are 2'-deoxy-2'-fluoro pyrimidine nucleotides or alternately a plurality of pyrimidine nucleotides are 2'-deoxy-2'-fluoro pyrimidine nucleotides), and for example where one or more purine nucleotides present in the sense region are selected from the group consisting of 2'-deoxy nucleotides, locked nucleic
20 acid (LNA) nucleotides, 2'-methoxyethyl nucleotides, 4'-thionucleotides; and 2'-O-methyl nucleotides (e.g., wherein all purine nucleotides are selected from the group consisting of 2'-deoxy nucleotides, locked nucleic acid (LNA) nucleotides, 2'-methoxyethyl nucleotides, 4'-thionucleotides, and 2'-O-methyl nucleotides or alternately a plurality of purine nucleotides are selected from the group consisting of 2'-deoxy
25 nucleotides, locked nucleic acid (LNA) nucleotides, 2'-methoxyethyl nucleotides, 4'-thionucleotides, and 2'-O-methyl nucleotides), and wherein inverted deoxy abasic modifications are optionally present at the 3'-end, the 5'-end, or both of the 3' and 5'-ends of the sense region, the sense region optionally further comprising a 3'-terminal overhang having about 1 to about 4 (e.g., about 1, 2, 3, or 4) 2'-deoxyribonucleotides; and wherein
30 the chemically-modified short interfering nucleic acid molecule comprises an antisense region, where one or more pyrimidine nucleotides present in the antisense region are 2'-deoxy-2'-fluoro pyrimidine nucleotides (e.g., wherein all pyrimidine nucleotides are 2'-deoxy-2'-fluoro pyrimidine nucleotides or alternately a plurality of pyrimidine

nucleotides are 2'-deoxy-2'-fluoro pyrimidine nucleotides), and wherein one or more purine nucleotides present in the antisense region are selected from the group consisting of 2'-deoxy nucleotides, locked nucleic acid (LNA) nucleotides, 2'-methoxyethyl nucleotides, 4'-thionucleotides, and 2'-O-methyl nucleotides (e.g., wherein all purine nucleotides are selected from the group consisting of 2'-deoxy nucleotides, locked nucleic acid (LNA) nucleotides, 2'-methoxyethyl nucleotides, 4'-thionucleotides, and 2'-O-methyl nucleotides or alternately a plurality of purine nucleotides are selected from the group consisting of 2'-deoxy nucleotides, locked nucleic acid (LNA) nucleotides, 2'-methoxyethyl nucleotides, 4'-thionucleotides, and 2'-O-methyl nucleotides), and a terminal cap modification, such as any modification described herein or shown in Figure 10, that is optionally present at the 3'-end, the 5'-end, or both of the 3' and 5'-ends of the antisense sequence, the antisense region optionally further comprising a 3'-terminal nucleotide overhang having about 1 to about 4 (e.g., about 1, 2, 3, or 4) 2'-deoxynucleotides, wherein the overhang nucleotides can further comprise one or more (e.g., 1, 2, 3, or 4) phosphorothioate internucleotide linkages.

In another embodiment, any modified nucleotides present in the siNA molecules of the invention, preferably in the antisense strand of the siNA molecules of the invention, but also optionally in the sense and/or both antisense and sense strands, comprise modified nucleotides having properties or characteristics similar to naturally occurring ribonucleotides. For example, the invention features siNA molecules including modified nucleotides having a Northern conformation (e.g., Northern pseudorotation cycle, see for example Saenger, *Principles of Nucleic Acid Structure*, Springer-Verlag ed., 1984). As such, chemically modified nucleotides present in the siNA molecules of the invention, preferably in the antisense strand of the siNA molecules of the invention, but also optionally in the sense and/or both antisense and sense strands, are resistant to nuclease degradation while at the same time maintaining the capacity to mediate RNAi. Non-limiting examples of nucleotides having a northern configuration include locked nucleic acid (LNA) nucleotides (e.g., 2'-O,4'-C-methylene-(D-ribofuranosyl) nucleotides); 2'-methoxyethoxy (MOE) nucleotides; 2'-methyl-thio-ethyl, 2'-deoxy-2'-fluoro nucleotides, 2'-deoxy-2'-chloro nucleotides, 2'-azido nucleotides, and 2'-O-methyl nucleotides.

In one embodiment, the invention features a chemically-modified short interfering nucleic acid molecule (siNA) capable of mediating RNA interference (RNAi) against a HCV inside a cell or reconstituted *in vitro* system, wherein the chemical modification comprises a conjugate covalently attached to the chemically-modified siNA molecule. In another embodiment, the conjugate is covalently attached to the chemically-modified siNA molecule via a biodegradable linker. In one embodiment, the conjugate molecule is attached at the 3'-end of either the sense strand, the antisense strand, or both strands of the chemically-modified siNA molecule. In another embodiment, the conjugate molecule is attached at the 5'-end of either the sense strand, the antisense strand, or both strands of the chemically-modified siNA molecule. In yet another embodiment, the conjugate molecule is attached both the 3'-end and 5'-end of either the sense strand, the antisense strand, or both strands of the chemically-modified siNA molecule, or any combination thereof. In one embodiment, a conjugate molecule of the invention comprises a molecule that facilitates delivery of a chemically-modified siNA molecule into a biological system, such as a cell. In another embodiment, the conjugate molecule attached to the chemically-modified siNA molecule is a poly ethylene glycol, human serum albumin, or a ligand for a cellular receptor that can mediate cellular uptake. Examples of specific conjugate molecules contemplated by the instant invention that can be attached to chemically-modified siNA molecules are described in Vargeese *et al.*, U.S. Serial No. 10/201,394, incorporated by reference herein. The type of conjugates used and the extent of conjugation of siNA molecules of the invention can be evaluated for improved pharmacokinetic profiles, bioavailability, and/or stability of siNA constructs while at the same time maintaining the ability of the siNA to mediate RNAi activity. As such, one skilled in the art can screen siNA constructs that are modified with various conjugates to determine whether the siNA conjugate complex possesses improved properties while maintaining the ability to mediate RNAi, for example in animal models as are generally known in the art.

In one embodiment, the invention features a short interfering nucleic acid (siNA) molecule of the invention, wherein the siNA further comprises a nucleotide, non-nucleotide, or mixed nucleotide/non-nucleotide linker that joins the sense region of the siNA to the antisense region of the siNA. In one embodiment, a nucleotide linker of the invention can be a linker of ≥ 2 nucleotides in length, for example 3, 4, 5, 6, 7, 8, 9, or 10 nucleotides in length. In another embodiment, the nucleotide linker can be a nucleic acid

aptamer. By "aptamer" or "nucleic acid aptamer" as used herein is meant a nucleic acid molecule that binds specifically to a target molecule wherein the nucleic acid molecule has sequence that comprises a sequence recognized by the target molecule in its natural setting. Alternately, an aptamer can be a nucleic acid molecule that binds to a target molecule where the target molecule does not naturally bind to a nucleic acid. The target molecule can be any molecule of interest. For example, the aptamer can be used to bind to a ligand-binding domain of a protein, thereby preventing interaction of the naturally occurring ligand with the protein. This is a non-limiting example and those in the art will recognize that other embodiments can be readily generated using techniques generally known in the art. (See, for example, Gold *et al.*, 1995, *Annu. Rev. Biochem.*, 64, 763; Brody and Gold, 2000, *J. Biotechnol.*, 74, 5; Sun, 2000, *Curr. Opin. Mol. Ther.*, 2, 100; Kusser, 2000, *J. Biotechnol.*, 74, 27; Hermann and Patel, 2000, *Science*, 287, 820; and Jayasena, 1999, *Clinical Chemistry*, 45, 1628.)

In yet another embodiment, a non-nucleotide linker of the invention comprises abasic nucleotide, polyether, polyamine, polyamide, peptide, carbohydrate, lipid, polyhydrocarbon, or other polymeric compounds (e.g. polyethylene glycols such as those having between 2 and 100 ethylene glycol units). Specific examples include those described by Seela and Kaiser, *Nucleic Acids Res.* 1990, 18:6353 and *Nucleic Acids Res.* 1987, 15:3113; Cload and Schepartz, *J. Am. Chem. Soc.* 1991, 113:6324; Richardson and Schepartz, *J. Am. Chem. Soc.* 1991, 113:5109; Ma *et al.*, *Nucleic Acids Res.* 1993, 21:2585 and *Biochemistry* 1993, 32:1751; Durand *et al.*, *Nucleic Acids Res.* 1990, 18:6353; McCurdy *et al.*, *Nucleosides & Nucleotides* 1991, 10:287; Jschke *et al.*, *Tetrahedron Lett.* 1993, 34:301; Ono *et al.*, *Biochemistry* 1991, 30:9914; Arnold *et al.*, International Publication No. WO 89/02439; Usman *et al.*, International Publication No. WO 95/06731; Dudycz *et al.*, International Publication No. WO 95/11910 and Ferentz and Verdine, *J. Am. Chem. Soc.* 1991, 113:4000, all hereby incorporated by reference herein. A "non-nucleotide" further means any group or compound that can be incorporated into a nucleic acid chain in the place of one or more nucleotide units, including either sugar and/or phosphate substitutions, and allows the remaining bases to exhibit their enzymatic activity. The group or compound can be abasic in that it does not contain a commonly recognized nucleotide base, such as adenosine, guanine, cytosine, uracil or thymine, for example at the C1 position of the sugar.

In one embodiment, the invention features a short interfering nucleic acid (siNA) molecule capable of mediating RNA interference (RNAi) inside a cell or reconstituted in vitro system, wherein one or both strands of the siNA molecule that are assembled from two separate oligonucleotides do not comprise any ribonucleotides. For example, a siNA molecule can be assembled from a single oligonucleotide where the sense and antisense regions of the siNA comprise separate oligonucleotides not having any ribonucleotides (e.g., nucleotides having a 2'-OH group) present in the oligonucleotides. In another example, a siNA molecule can be assembled from a single oligonucleotide where the sense and antisense regions of the siNA are linked or circularized by a nucleotide or non-nucleotide linker as described herein, wherein the oligonucleotide does not have any ribonucleotides (e.g., nucleotides having a 2'-OH group) present in the oligonucleotide. Applicant has surprisingly found that the presense of ribonucleotides (e.g., nucleotides having a 2'-hydroxyl group) within the siNA molecule is not required or essential to support RNAi activity. As such, in one embodiment, all positions within the siNA can include chemically modified nucleotides and/or non-nucleotides such as nucleotides and or non-nucleotides having Formula I, II, III, IV, V, VI, or VII or any combination thereof to the extent that the ability of the siNA molecule to support RNAi activity in a cell is maintained.

In one embodiment, a siNA molecule of the invention is a single stranded siNA molecule that mediates RNAi activity in a cell or reconstituted in vitro system, wherein the siNA molecule comprises a single stranded polynucleotide having complementarity to a target nucleic acid sequence. In another embodiment, the single stranded siNA molecule of the invention comprises a 5'-terminal phosphate group. In another embodiment, the single stranded siNA molecule of the invention comprises a 5'-terminal phosphate group and a 3'-terminal phosphate group (e.g., a 2',3'-cyclic phosphate). In another embodiment, the single stranded siNA molecule of the invention comprises about 19 to about 29 nucleotides. In yet another embodiment, the single stranded siNA molecule of the invention comprises one or more chemically modified nucleotides or non-nucleotides described herein. For example, all the positions within the siNA molecule can include chemically-modified nucleotides such as nucleotides having any of Formulae I-VII, or any combination thereof to the extent that the ability of the siNA molecule to support RNAi activity in a cell is maintained.

In one embodiment, a siNA molecule of the invention is a single stranded siNA molecule that mediates RNAi activity in a cell or reconstituted in vitro system, wherein the siNA molecule comprises a single stranded polynucleotide having complementarity to a target nucleic acid sequence, and wherein one or more pyrimidine nucleotides present in the siNA are 2'-deoxy-2'-fluoro pyrimidine nucleotides (e.g., wherein all pyrimidine nucleotides are 2'-deoxy-2'-fluoro pyrimidine nucleotides or alternately a plurality of pyrimidine nucleotides are 2'-deoxy-2'-fluoro pyrimidine nucleotides), and wherein any purine nucleotides present in the antisense region are 2'-O-methyl purine nucleotides (e.g., wherein all purine nucleotides are 2'-O-methyl purine nucleotides or alternately a plurality of purine nucleotides are 2'-O-methyl purine nucleotides), and a terminal cap modification, such as any modification described herein or shown in Figure 10, that is optionally present at the 3'-end, the 5'-end, or both of the 3' and 5'-ends of the antisense sequence, the siNA optionally further comprising about 1 to about 4 (e.g., about 1, 2, 3, or 4) terminal 2'-deoxynucleotides at the 3'-end of the siNA molecule, wherein the terminal nucleotides can further comprise one or more (e.g., 1, 2, 3, or 4) phosphorothioate internucleotide linkages, and wherein the siNA optionally further comprises a terminal phosphate group, such as a 5'-terminal phosphate group.

In one embodiment, a siNA molecule of the invention is a single stranded siNA molecule that mediates RNAi activity in a cell or reconstituted in vitro system, wherein the siNA molecule comprises a single stranded polynucleotide having complementarity to a target nucleic acid sequence, and wherein one or more pyrimidine nucleotides present in the siNA are 2'-deoxy-2'-fluoro pyrimidine nucleotides (e.g., wherein all pyrimidine nucleotides are 2'-deoxy-2'-fluoro pyrimidine nucleotides or alternately a plurality of pyrimidine nucleotides are 2'-deoxy-2'-fluoro pyrimidine nucleotides), and wherein any purine nucleotides present in the antisense region are 2'-deoxy purine nucleotides (e.g., wherein all purine nucleotides are 2'-deoxy purine nucleotides or alternately a plurality of purine nucleotides are 2'-deoxy purine nucleotides), and a terminal cap modification, such as any modification described herein or shown in Figure 10, that is optionally present at the 3'-end, the 5'-end, or both of the 3' and 5'-ends of the antisense sequence, the siNA optionally further comprising about 1 to about 4 (e.g., about 1, 2, 3, or 4) terminal 2'-deoxynucleotides at the 3'-end of the siNA molecule, wherein the terminal nucleotides can further comprise one or more (e.g., 1, 2, 3, or 4)

phosphorothioate internucleotide linkages, and wherein the siNA optionally further comprises a terminal phosphate group, such as a 5'-terminal phosphate group.

In one embodiment, a siNA molecule of the invention is a single stranded siNA molecule that mediates RNAi activity in a cell or reconstituted in vitro system, wherein the siNA molecule comprises a single stranded polynucleotide having complementarity to a target nucleic acid sequence, and wherein one or more pyrimidine nucleotides present in the siNA are 2'-deoxy-2'-fluoro pyrimidine nucleotides (e.g., wherein all pyrimidine nucleotides are 2'-deoxy-2'-fluoro pyrimidine nucleotides or alternately a plurality of pyrimidine nucleotides are 2'-deoxy-2'-fluoro pyrimidine nucleotides), and wherein any purine nucleotides present in the antisense region are locked nucleic acid (LNA) nucleotides (e.g., wherein all purine nucleotides are LNA nucleotides or alternately a plurality of purine nucleotides are LNA nucleotides), and a terminal cap modification, such as any modification described herein or shown in Figure 10, that is optionally present at the 3'-end, the 5'-end, or both of the 3' and 5'-ends of the antisense sequence, the siNA optionally further comprising about 1 to about 4 (e.g., about 1, 2, 3, or 4) terminal 2'-deoxynucleotides at the 3'-end of the siNA molecule, wherein the terminal nucleotides can further comprise one or more (e.g., 1, 2, 3, or 4) phosphorothioate internucleotide linkages, and wherein the siNA optionally further comprises a terminal phosphate group, such as a 5'-terminal phosphate group.

In one embodiment, a siNA molecule of the invention is a single stranded siNA molecule that mediates RNAi activity in a cell or reconstituted in vitro system, wherein the siNA molecule comprises a single stranded polynucleotide having complementarity to a target nucleic acid sequence, and wherein one or more pyrimidine nucleotides present in the siNA are 2'-deoxy-2'-fluoro pyrimidine nucleotides (e.g., wherein all pyrimidine nucleotides are 2'-deoxy-2'-fluoro pyrimidine nucleotides or alternately a plurality of pyrimidine nucleotides are 2'-deoxy-2'-fluoro pyrimidine nucleotides), and wherein any purine nucleotides present in the antisense region are 2'-methoxyethyl purine nucleotides (e.g., wherein all purine nucleotides are 2'-methoxyethyl purine nucleotides or alternately a plurality of purine nucleotides are 2'-methoxyethyl purine nucleotides), and a terminal cap modification, such as any modification described herein or shown in Figure 10, that is optionally present at the 3'-end, the 5'-end, or both of the 3' and 5'-ends of the antisense sequence, the siNA optionally further comprising about 1

to about 4 (e.g., about 1, 2, 3, or 4) terminal 2'-deoxynucleotides at the 3'-end of the siNA molecule, wherein the terminal nucleotides can further comprise one or more (e.g., 1, 2, 3, or 4) phosphorothioate internucleotide linkages, and wherein the siNA optionally further comprises a terminal phosphate group, such as a 5'-terminal phosphate group.

5 In another embodiment, any modified nucleotides present in the single stranded siNA molecules of the invention comprise modified nucleotides having properties or characteristics similar to naturally occurring ribonucleotides. For example, the invention features siNA molecules including modified nucleotides having a Northern conformation (e.g., Northern pseudorotation cycle, see for example Saenger, *Principles of Nucleic Acid*
10 *Structure*, Springer-Verlag ed., 1984). As such, chemically modified nucleotides present in the single stranded siNA molecules of the invention are preferably resistant to nuclease degradation while at the same time maintaining the capacity to mediate RNAi.

In one embodiment, the invention features a method for modulating the expression of a HCV gene within a cell comprising: (a) synthesizing a siNA molecule of the
15 invention, which can be chemically-modified, wherein one of the siNA strands comprises a sequence complementary to RNA of the HCV gene; and (b) introducing the siNA molecule into a cell under conditions suitable to modulate the expression of the HCV gene in the cell.

In one embodiment, the invention features a method for modulating the expression
20 of a HCV gene within a cell comprising: (a) synthesizing a siNA molecule of the invention, which can be chemically-modified, wherein one of the siNA strands comprises a sequence complementary to RNA of the HCV gene and wherein the sense strand sequence of the siNA comprises a sequence identical to the sequence of the target RNA; and (b) introducing the siNA molecule into a cell under conditions suitable to
25 modulate the expression of the HCV gene in the cell.

In another embodiment, the invention features a method for modulating the expression of more than one HCV gene within a cell comprising: (a) synthesizing siNA molecules of the invention, which can be chemically-modified, wherein one of the siNA strands comprises a sequence complementary to RNA of the HCV genes; and (b)
30 introducing the siNA molecules into a cell under conditions suitable to modulate the expression of the HCV genes in the cell.

In another embodiment, the invention features a method for modulating the expression of more than one HCV gene within a cell comprising: (a) synthesizing a siNA molecule of the invention, which can be chemically-modified, wherein one of the siNA strands comprises a sequence complementary to RNA of the HCV gene and
5 wherein the sense strand sequence of the siNA comprises a sequence identical to the sequence of the target RNA; and (b) introducing the siNA molecules into a cell under conditions suitable to modulate the expression of the HCV genes in the cell.

In one embodiment, the invention features a method of modulating the expression of a HCV gene in a tissue explant comprising: (a) synthesizing a siNA molecule of the
10 invention, which can be chemically-modified, wherein one of the siNA strands comprises a sequence complementary to RNA of the HCV gene; and (b) introducing the siNA molecule into a cell of the tissue explant derived from a particular organism under conditions suitable to modulate the expression of the HCV gene in the tissue explant. In another embodiment, the method further comprises introducing the tissue explant back
15 into the organism the tissue was derived from or into another organism under conditions suitable to modulate the expression of the HCV gene in that organism.

In one embodiment, the invention features a method of modulating the expression of a HCV gene in a tissue explant comprising: (a) synthesizing a siNA molecule of the invention, which can be chemically-modified, wherein one of the siNA strands
20 comprises a sequence complementary to RNA of the HCV gene and wherein the sense strand sequence of the siNA comprises a sequence identical to the sequence of the target RNA; and (b) introducing the siNA molecule into a cell of the tissue explant derived from a particular organism under conditions suitable to modulate the expression of the HCV gene in the tissue explant. In another embodiment, the method further comprises
25 introducing the tissue explant back into the organism the tissue was derived from or into another organism under conditions suitable to modulate the expression of the HCV gene in that organism.

In another embodiment, the invention features a method of modulating the expression of more than one HCV gene in a tissue explant comprising: (a) synthesizing
30 siNA molecules of the invention, which can be chemically-modified, wherein one of the siNA strands comprises a sequence complementary to RNA of the HCV genes; and (b)

introducing the siNA molecules into a cell of the tissue explant derived from a particular organism under conditions suitable to modulate the expression of the HCV genes in the tissue explant. In another embodiment, the method further comprises introducing the tissue explant back into the organism the tissue was derived from or into another
5 organism under conditions suitable to modulate the expression of the HCV genes in that organism.

In one embodiment, the invention features a method of modulating the expression of a HCV gene in an organism comprising: (a) synthesizing a siNA molecule of the invention, which can be chemically-modified, wherein one of the siNA strands
10 comprises a sequence complementary to RNA of the HCV gene; and (b) introducing the siNA molecule into the organism under conditions suitable to modulate the expression of the HCV gene in the organism.

In another embodiment, the invention features a method of modulating the expression of more than one HCV gene in an organism comprising: (a) synthesizing
15 siNA molecules of the invention, which can be chemically-modified, wherein one of the siNA strands comprises a sequence complementary to RNA of the HCV genes; and (b) introducing the siNA molecules into the organism under conditions suitable to modulate the expression of the HCV genes in the organism.

In one embodiment, the invention features a method for modulating the expression
20 of a HCV gene within a cell comprising: (a) synthesizing a siNA molecule of the invention, which can be chemically-modified, wherein the siNA comprises a single stranded sequence having complementarity to RNA of the HCV gene; and (b) introducing the siNA molecule into a cell under conditions suitable to modulate the expression of the HCV gene in the cell.

25 In another embodiment, the invention features a method for modulating the expression of more than one HCV gene within a cell comprising: (a) synthesizing siNA molecules of the invention, which can be chemically-modified, wherein the siNA comprises a single stranded sequence having complementarity to RNA of the HCV gene; and (b) contacting the siNA molecule with a cell in vitro or in vivo under conditions
30 suitable to modulate the expression of the HCV genes in the cell.

In one embodiment, the invention features a method of modulating the expression of a HCV gene in a tissue explant comprising: (a) synthesizing a siNA molecule of the invention, which can be chemically-modified, wherein the siNA comprises a single stranded sequence having complementarity to RNA of the HCV gene; and (b) contacting
5 the siNA molecule with a cell of the tissue explant derived from a particular organism under conditions suitable to modulate the expression of the HCV gene in the tissue explant. In another embodiment, the method further comprises introducing the tissue explant back into the organism the tissue was derived from or into another organism under conditions suitable to modulate the expression of the HCV gene in that organism.

10 In another embodiment, the invention features a method of modulating the expression of more than one HCV gene in a tissue explant comprising: (a) synthesizing siNA molecules of the invention, which can be chemically-modified, wherein the siNA comprises a single stranded sequence having complementarity to RNA of the HCV gene; and (b) introducing the siNA molecules into a cell of the tissue explant derived from a
15 particular organism under conditions suitable to modulate the expression of the HCV genes in the tissue explant. In another embodiment, the method further comprises introducing the tissue explant back into the organism the tissue was derived from or into another organism under conditions suitable to modulate the expression of the HCV genes in that organism.

20 In one embodiment, the invention features a method of modulating the expression of a HCV gene in an organism comprising: (a) synthesizing a siNA molecule of the invention, which can be chemically-modified, wherein the siNA comprises a single stranded sequence having complementarity to RNA of the HCV gene; and (b) introducing the siNA molecule into the organism under conditions suitable to modulate
25 the expression of the HCV gene in the organism.

In another embodiment, the invention features a method of modulating the expression of more than one HCV gene in an organism comprising: (a) synthesizing siNA molecules of the invention, which can be chemically-modified, wherein the siNA comprises a single stranded sequence having complementarity to RNA of the HCV gene;
30 and (b) introducing the siNA molecules into the organism under conditions suitable to modulate the expression of the HCV genes in the organism.

In one embodiment, the invention features a method of modulating the expression of a HCV gene in an organism comprising contacting the organism with a siNA molecule of the invention under conditions suitable to modulate the expression of the HCV gene in the organism.

5 In another embodiment, the invention features a method of modulating the expression of more than one HCV gene in an organism comprising contacting the organism with one or more siNA molecules of the invention under conditions suitable to modulate the expression of the HCV genes in the organism.

The siNA molecules of the invention can be designed to inhibit target (HCV) gene
10 expression through RNAi targeting of a variety of RNA molecules. In one embodiment, the siNA molecules of the invention are used to target various RNAs corresponding to a target gene. Non-limiting examples of such RNAs include messenger RNA (mRNA), alternate RNA splice variants of target gene(s), post-transcriptionally modified RNA of target gene(s), pre-mRNA of target gene(s), and/or RNA templates. If alternate splicing
15 produces a family of transcripts that are distinguished by usage of appropriate exons, the instant invention can be used to inhibit gene expression through the appropriate exons to specifically inhibit or to distinguish among the functions of gene family members. For example, a protein that contains an alternatively spliced transmembrane domain can be expressed in both membrane bound and secreted forms. Use of the invention to target
20 the exon containing the transmembrane domain can be used to determine the functional consequences of pharmaceutical targeting of membrane bound as opposed to the secreted form of the protein. Non-limiting examples of applications of the invention relating to targeting these RNA molecules include therapeutic pharmaceutical applications, pharmaceutical discovery applications, molecular diagnostic and gene function
25 applications, and gene mapping, for example using single nucleotide polymorphism mapping with siNA molecules of the invention. Such applications can be implemented using known gene sequences or from partial sequences available from an expressed sequence tag (EST).

In another embodiment, the siNA molecules of the invention are used to target
30 conserved sequences corresponding to a gene family or gene families such as HCV family genes. As such, siNA molecules targeting multiple HCV targets can provide

increased therapeutic effect. In addition, siNA can be used to characterize pathways of gene function in a variety of applications. For example, the present invention can be used to inhibit the activity of target gene(s) in a pathway to determine the function of uncharacterized gene(s) in gene function analysis, mRNA function analysis, or translational analysis. The invention can be used to determine potential target gene pathways involved in various diseases and conditions toward pharmaceutical development. The invention can be used to understand pathways of gene expression involved in, for example, the progression and/or maintenance of HCV infection, liver failure, hepatocellular carcinoma, cirrhosis and other indications that can respond to the level of HCV in a cell or tissue.

In one embodiment, siNA molecule(s) and/or methods of the invention are used to inhibit the expression of gene(s) that encode RNA referred to by Genbank Accession, for example HCV genes encoding RNA sequence(s) referred to herein by Genbank Accession number, for example Genbank Accession Nos. shown in Table I.

In one embodiment, the invention features a method comprising: (a) generating a library of siNA constructs having a predetermined complexity; and (b) assaying the siNA constructs of (a) above, under conditions suitable to determine RNAi target sites within the target RNA sequence. In another embodiment, the siNA molecules of (a) have strands of a fixed length, for example, about 23 nucleotides in length. In yet another embodiment, the siNA molecules of (a) are of differing length, for example having strands of about 19 to about 25 (*e.g.*, about 19, 20, 21, 22, 23, 24, or 25) nucleotides in length. In one embodiment, the assay can comprise a reconstituted *in vitro* siNA assay as described herein. In another embodiment, the assay can comprise a cell culture system in which target RNA is expressed. In another embodiment, fragments of target RNA are analyzed for detectable levels of cleavage, for example by gel electrophoresis, northern blot analysis, or RNase protection assays, to determine the most suitable target site(s) within the target RNA sequence. The target RNA sequence can be obtained as is known in the art, for example, by cloning and/or transcription for *in vitro* systems, and by cellular expression in *in vivo* systems.

In one embodiment, the invention features a method comprising: (a) generating a randomized library of siNA constructs having a predetermined complexity, such as of 4^N ,

where N represents the number of base paired nucleotides in each of the siNA construct strands (eg. for a siNA construct having 21 nucleotide sense and antisense strands with 19 base pairs, the complexity would be 4^{19}); and (b) assaying the siNA constructs of (a) above, under conditions suitable to determine RNAi target sites within the target HCV RNA sequence. In another embodiment, the siNA molecules of (a) have strands of a fixed length, for example about 23 nucleotides in length. In yet another embodiment, the siNA molecules of (a) are of differing length, for example having strands of about 19 to about 25 (e.g., about 19, 20, 21, 22, 23, 24, or 25) nucleotides in length. In one embodiment, the assay can comprise a reconstituted *in vitro* siNA assay as described in Example 6 herein. In another embodiment, the assay can comprise a cell culture system in which target RNA is expressed. In another embodiment, fragments of HCV RNA are analyzed for detectable levels of cleavage, for example by gel electrophoresis, northern blot analysis, or RNase protection assays, to determine the most suitable target site(s) within the target HCV RNA sequence. The target HCV RNA sequence can be obtained as is known in the art, for example, by cloning and/or transcription for *in vitro* systems, and by cellular expression in *in vivo* systems.

In another embodiment, the invention features a method comprising: (a) analyzing the sequence of a RNA target encoded by a target gene; (b) synthesizing one or more sets of siNA molecules having sequence complementary to one or more regions of the RNA of (a); and (c) assaying the siNA molecules of (b) under conditions suitable to determine RNAi targets within the target RNA sequence. In one embodiment, the siNA molecules of (b) have strands of a fixed length, for example about 23 nucleotides in length. In another embodiment, the siNA molecules of (b) are of differing length, for example having strands of about 19 to about 25 (e.g., about 19, 20, 21, 22, 23, 24, or 25) nucleotides in length. In one embodiment, the assay can comprise a reconstituted *in vitro* siNA assay as described herein. In another embodiment, the assay can comprise a cell culture system in which target RNA is expressed. Fragments of target RNA are analyzed for detectable levels of cleavage, for example by gel electrophoresis, northern blot analysis, or RNase protection assays, to determine the most suitable target site(s) within the target RNA sequence. The target RNA sequence can be obtained as is known in the art, for example, by cloning and/or transcription for *in vitro* systems, and by expression in *in vivo* systems.

By "target site" is meant a sequence within a target RNA that is "targeted" for cleavage mediated by a siNA construct which contains sequences within its antisense region that are complementary to the target sequence.

By "detectable level of cleavage" is meant cleavage of target RNA (and formation of cleaved product RNAs) to an extent sufficient to discern cleavage products above the background of RNAs produced by random degradation of the target RNA. Production of cleavage products from 1-5% of the target RNA is sufficient to detect above the background for most methods of detection.

In one embodiment, the invention features a composition comprising a siNA molecule of the invention, which can be chemically-modified, in a pharmaceutically acceptable carrier or diluent. In another embodiment, the invention features a pharmaceutical composition comprising siNA molecules of the invention, which can be chemically-modified, targeting one or more genes in a pharmaceutically acceptable carrier or diluent. In another embodiment, the invention features a method for treating or preventing a disease or condition in a subject, comprising administering to the subject a composition of the invention under conditions suitable for the treatment or prevention of the disease or condition in the subject, alone or in conjunction with one or more other therapeutic compounds. In yet another embodiment, the invention features a method for reducing or preventing tissue rejection in a subject comprising administering to the subject a composition of the invention under conditions suitable for the reduction or prevention of tissue rejection in the subject.

In another embodiment, the invention features a method for validating a HCV gene target comprising: (a) synthesizing a siNA molecule of the invention, which can be chemically-modified, wherein one of the siNA strands comprises a sequence complementary to RNA of a HCV target gene; (b) introducing the siNA molecule into a cell, tissue, or organism under conditions suitable for modulating expression of the HCV target gene in the cell, tissue, or organism; and (c) determining the function of the gene by assaying for any phenotypic change in the cell, tissue, or organism.

In another embodiment, the invention features a method for validating a HCV gene target comprising: (a) synthesizing a siNA molecule of the invention, which can be chemically-modified, wherein one of the siNA strands includes a sequence

complementary to RNA of a HCV target gene; (b) introducing the siNA molecule into a biological system under conditions suitable for modulating expression of the HCV target gene in the biological system; and (c) determining the function of the gene by assaying for any phenotypic change in the biological system.

5 By "biological system" is meant, material, in a purified or unpurified form, from biological sources, including but not limited to human, animal, plant, insect, bacterial, viral or other sources, wherein the system comprises the components required for RNAi activity. The term "biological system" includes, for example, a cell, tissue, or organism, or extract thereof. The term biological system also includes reconstituted RNAi systems
10 that can be used in an *in vitro* setting.

By "phenotypic change" is meant any detectable change to a cell that occurs in response to contact or treatment with a nucleic acid molecule of the invention (e.g., siNA). Such detectable changes include, but are not limited to, changes in shape, size, proliferation, motility, protein expression or RNA expression or other physical or
15 chemical changes as can be assayed by methods known in the art. The detectable change can also include expression of reporter genes/molecules such as Green Florescent Protein (GFP) or various tags that are used to identify an expressed protein or any other cellular component that can be assayed.

In one embodiment, the invention features a kit containing a siNA molecule of the
20 invention, which can be chemically-modified, that can be used to modulate the expression of a HCV target gene in a cell, tissue, or organism. In another embodiment, the invention features a kit containing more than one siNA molecule of the invention, which can be chemically-modified, that can be used to modulate the expression of more than one HCV target gene in a cell, tissue, or organism.

25 In one embodiment, the invention features a cell containing one or more siNA molecules of the invention, which can be chemically-modified. In another embodiment, the cell containing a siNA molecule of the invention is a mammalian cell. In yet another embodiment, the cell containing a siNA molecule of the invention is a human cell.

In one embodiment, the synthesis of a siNA molecule of the invention, which can
30 be chemically-modified, comprises: (a) synthesis of two complementary strands of the

siNA molecule; (b) annealing the two complementary strands together under conditions suitable to obtain a double-stranded siNA molecule. In another embodiment, synthesis of the two complementary strands of the siNA molecule is by solid phase oligonucleotide synthesis. In yet another embodiment, synthesis of the two complementary strands of the
5 siNA molecule is by solid phase tandem oligonucleotide synthesis.

In one embodiment, the invention features a method for synthesizing a siNA duplex molecule comprising: (a) synthesizing a first oligonucleotide sequence strand of the siNA molecule, wherein the first oligonucleotide sequence strand comprises a cleavable linker molecule that can be used as a scaffold for the synthesis of the second
10 oligonucleotide sequence strand of the siNA; (b) synthesizing the second oligonucleotide sequence strand of siNA on the scaffold of the first oligonucleotide sequence strand, wherein the second oligonucleotide sequence strand further comprises a chemical moiety than can be used to purify the siNA duplex; (c) cleaving the linker molecule of (a) under conditions suitable for the two siNA oligonucleotide strands to hybridize and form a
15 stable duplex; and (d) purifying the siNA duplex utilizing the chemical moiety of the second oligonucleotide sequence strand. In one embodiment, cleavage of the linker molecule in (c) above takes place during deprotection of the oligonucleotide, for example under hydrolysis conditions using an alkylamine base such as methylamine. In one embodiment, the method of synthesis comprises solid phase synthesis on a solid support
20 such as controlled pore glass (CPG) or polystyrene, wherein the first sequence of (a) is synthesized on a cleavable linker, such as a succinyl linker, using the solid support as a scaffold. The cleavable linker in (a) used as a scaffold for synthesizing the second strand can comprise similar reactivity as the solid support derivatized linker, such that cleavage of the solid support derivatized linker and the cleavable linker of (a) takes place
25 concomitantly. In another embodiment, the chemical moiety of (b) that can be used to isolate the attached oligonucleotide sequence comprises a trityl group, for example a dimethoxytrityl group, which can be employed in a trityl-on synthesis strategy as described herein. In yet another embodiment, the chemical moiety, such as a dimethoxytrityl group, is removed during purification, for example, using acidic
30 conditions.

In a further embodiment, the method for siNA synthesis is a solution phase synthesis or hybrid phase synthesis wherein both strands of the siNA duplex are

synthesized in tandem using a cleavable linker attached to the first sequence which acts a scaffold for synthesis of the second sequence. Cleavage of the linker under conditions suitable for hybridization of the separate siNA sequence strands results in formation of the double-stranded siNA molecule.

5 In another embodiment, the invention features a method for synthesizing a siNA duplex molecule comprising: (a) synthesizing one oligonucleotide sequence strand of the siNA molecule, wherein the sequence comprises a cleavable linker molecule that can be used as a scaffold for the synthesis of another oligonucleotide sequence; (b) synthesizing a second oligonucleotide sequence having complementarity to the first
10 sequence strand on the scaffold of (a), wherein the second sequence comprises the other strand of the double-stranded siNA molecule and wherein the second sequence further comprises a chemical moiety than can be used to isolate the attached oligonucleotide sequence; (c) purifying the product of (b) utilizing the chemical moiety of the second oligonucleotide sequence strand under conditions suitable for isolating the full-length
15 sequence comprising both siNA oligonucleotide strands connected by the cleavable linker and under conditions suitable for the two siNA oligonucleotide strands to hybridize and form a stable duplex. In one embodiment, cleavage of the linker molecule in (c) above takes place during deprotection of the oligonucleotide, for example under hydrolysis conditions. In another embodiment, cleavage of the linker molecule in (c)
20 above takes place after deprotection of the oligonucleotide. In another embodiment, the method of synthesis comprises solid phase synthesis on a solid support such as controlled pore glass (CPG) or polystyrene, wherein the first sequence of (a) is synthesized on a cleavable linker, such as a succinyl linker, using the solid support as a scaffold. The cleavable linker in (a) used as a scaffold for synthesizing the second strand can comprise
25 similar reactivity or differing reactivity as the solid support derivatized linker, such that cleavage of the solid support derivatized linker and the cleavable linker of (a) takes place either concomitantly or sequentially. In one embodiment, the chemical moiety of (b) that can be used to isolate the attached oligonucleotide sequence comprises a trityl group, for example a dimethoxytrityl group.

30 In another embodiment, the invention features a method for making a double-stranded siNA molecule in a single synthetic process comprising: (a) synthesizing an oligonucleotide having a first and a second sequence, wherein the first sequence is

complementary to the second sequence, and the first oligonucleotide sequence is linked to the second sequence via a cleavable linker, and wherein a terminal 5'-protecting group, for example, a 5'-O-dimethoxytrityl group (5'-O-DMT) remains on the oligonucleotide having the second sequence; (b) deprotecting the oligonucleotide whereby the
5 deprotection results in the cleavage of the linker joining the two oligonucleotide sequences; and (c) purifying the product of (b) under conditions suitable for isolating the double-stranded siNA molecule, for example using a trityl-on synthesis strategy as described herein.

In another embodiment, the method of synthesis of siNA molecules of the
10 invention comprises the teachings of Scaringe *et al.*, US Patent Nos. 5,889,136; 6,008,400; and 6,111,086, incorporated by reference herein in their entirety.

In one embodiment, the invention features siNA constructs that mediate RNAi against a HCV, wherein the siNA construct comprises one or more chemical modifications, for example, one or more chemical modifications having any of Formulae
15 I-VII or any combination thereof that increases the nuclease resistance of the siNA construct.

In another embodiment, the invention features a method for generating siNA molecules with increased nuclease resistance comprising (a) introducing nucleotides having any of Formula I-VII or any combination thereof into a siNA molecule, and (b)
20 assaying the siNA molecule of step (a) under conditions suitable for isolating siNA molecules having increased nuclease resistance.

In one embodiment, the invention features siNA constructs that mediate RNAi against a HCV, wherein the siNA construct comprises one or more chemical modifications described herein that modulates the binding affinity between the sense and
25 antisense strands of the siNA construct.

In another embodiment, the invention features a method for generating siNA molecules with increased binding affinity between the sense and antisense strands of the siNA molecule comprising (a) introducing nucleotides having any of Formula I-VII or any combination thereof into a siNA molecule, and (b) assaying the siNA molecule of

step (a) under conditions suitable for isolating siNA molecules having increased binding affinity between the sense and antisense strands of the siNA molecule.

In one embodiment, the invention features siNA constructs that mediate RNAi against a HCV, wherein the siNA construct comprises one or more chemical
5 modifications described herein that modulates the binding affinity between the antisense strand of the siNA construct and a complementary target RNA sequence within a cell.

In one embodiment, the invention features siNA constructs that mediate RNAi against a HCV, wherein the siNA construct comprises one or more chemical
10 modifications described herein that modulates the binding affinity between the antisense strand of the siNA construct and a complementary target DNA sequence within a cell.

In another embodiment, the invention features a method for generating siNA molecules with increased binding affinity between the antisense strand of the siNA molecule and a complementary target RNA sequence comprising (a) introducing
15 nucleotides having any of Formula I-VII or any combination thereof into a siNA molecule, and (b) assaying the siNA molecule of step (a) under conditions suitable for isolating siNA molecules having increased binding affinity between the antisense strand of the siNA molecule and a complementary target RNA sequence.

In another embodiment, the invention features a method for generating siNA molecules with increased binding affinity between the antisense strand of the siNA
20 molecule and a complementary target DNA sequence comprising (a) introducing nucleotides having any of Formula I-VII or any combination thereof into a siNA molecule, and (b) assaying the siNA molecule of step (a) under conditions suitable for isolating siNA molecules having increased binding affinity between the antisense strand of the siNA molecule and a complementary target DNA sequence.

25 In one embodiment, the invention features siNA constructs that mediate RNAi against a HCV, wherein the siNA construct comprises one or more chemical modifications described herein that modulate the polymerase activity of a cellular polymerase capable of generating additional endogenous siNA molecules having sequence homology to the chemically-modified siNA construct.

In another embodiment, the invention features a method for generating siNA molecules capable of mediating increased polymerase activity of a cellular polymerase capable of generating additional endogenous siNA molecules having sequence homology to a chemically-modified siNA molecule comprising (a) introducing nucleotides having
5 any of Formula I-VII or any combination thereof into a siNA molecule, and (b) assaying the siNA molecule of step (a) under conditions suitable for isolating siNA molecules capable of mediating increased polymerase activity of a cellular polymerase capable of generating additional endogenous siNA molecules having sequence homology to the chemically-modified siNA molecule.

10 In one embodiment, the invention features chemically-modified siNA constructs that mediate RNAi against a HCV in a cell, wherein the chemical modifications do not significantly effect the interaction of siNA with a target RNA molecule, DNA molecule and/or proteins or other factors that are essential for RNAi in a manner that would decrease the efficacy of RNAi mediated by such siNA constructs.

15 In another embodiment, the invention features a method for generating siNA molecules with improved RNAi activity against HCV comprising (a) introducing nucleotides having any of Formula I-VII or any combination thereof into a siNA molecule, and (b) assaying the siNA molecule of step (a) under conditions suitable for isolating siNA molecules having improved RNAi activity.

20 In yet another embodiment, the invention features a method for generating siNA molecules with improved RNAi activity against a HCV target RNA comprising (a) introducing nucleotides having any of Formula I-VII or any combination thereof into a siNA molecule, and (b) assaying the siNA molecule of step (a) under conditions suitable for isolating siNA molecules having improved RNAi activity against the target RNA.

25 In yet another embodiment, the invention features a method for generating siNA molecules with improved RNAi activity against a HCV target DNA comprising (a) introducing nucleotides having any of Formula I-VII or any combination thereof into a siNA molecule, and (b) assaying the siNA molecule of step (a) under conditions suitable for isolating siNA molecules having improved RNAi activity against the target DNA.

In one embodiment, the invention features siNA constructs that mediate RNAi against a HCV, wherein the siNA construct comprises one or more chemical modifications described herein that modulates the cellular uptake of the siNA construct.

In another embodiment, the invention features a method for generating siNA molecules against HCV with improved cellular uptake comprising (a) introducing
5 nucleotides having any of Formula I-VII or any combination thereof into a siNA molecule, and (b) assaying the siNA molecule of step (a) under conditions suitable for isolating siNA molecules having improved cellular uptake.

In one embodiment, the invention features siNA constructs that mediate RNAi
10 against a HCV, wherein the siNA construct comprises one or more chemical modifications described herein that increases the bioavailability of the siNA construct, for example, by attaching polymeric conjugates such as polyethyleneglycol or equivalent conjugates that improve the pharmacokinetics of the siNA construct, or by attaching conjugates that target specific tissue types or cell types *in vivo*. Non-limiting examples
15 of such conjugates are described in Vargeese *et al.*, U.S. Serial No. 10/201,394 incorporated by reference herein.

In one embodiment, the invention features a method for generating siNA molecules of the invention with improved bioavailability, comprising (a) introducing a conjugate into the structure of a siNA molecule, and (b) assaying the siNA molecule of
20 step (a) under conditions suitable for isolating siNA molecules having improved bioavailability. Such conjugates can include ligands for cellular receptors, such as peptides derived from naturally occurring protein ligands; protein localization sequences, including cellular ZIP code sequences; antibodies; nucleic acid aptamers; vitamins and other co-factors, such as folate and N-acetylgalactosamine; polymers, such as
25 polyethyleneglycol (PEG); phospholipids; polyamines, such as spermine or spermidine; and others.

In another embodiment, the invention features a method for generating siNA molecules of the invention with improved bioavailability comprising (a) introducing an excipient formulation to a siNA molecule, and (b) assaying the siNA molecule of step (a)
30 under conditions suitable for isolating siNA molecules having improved bioavailability.

Such excipients include polymers such as cyclodextrins, lipids, cationic lipids, polyamines, phospholipids, and others.

In another embodiment, the invention features a method for generating siNA molecules of the invention with improved bioavailability comprising (a) introducing
5 nucleotides having any of Formulae I-VII or any combination thereof into a siNA molecule, and (b) assaying the siNA molecule of step (a) under conditions suitable for isolating siNA molecules having improved bioavailability.

In another embodiment, polyethylene glycol (PEG) can be covalently attached to siNA compounds of the present invention. The attached PEG can be any molecular
10 weight, preferably from about 2,000 to about 50,000 daltons (Da).

The present invention can be used alone or as a component of a kit having at least one of the reagents necessary to carry out the *in vitro* or *in vivo* introduction of RNA to test samples and/or subjects. For example, preferred components of the kit include a siNA molecule of the invention and a vehicle that promotes introduction of the siNA into
15 cells of interest as described herein (e.g., using lipids and other methods of transfection known in the art, see for example Beigelman *et al.*, US 6,395,713). The kit can be used for target validation, such as in determining gene function and/or activity, or in drug optimization, and in drug discovery (see for example Usman *et al.*, USSN 60/402,996). Such a kit can also include instructions to allow a user of the kit to practice the invention.

20 The term "short interfering nucleic acid", "siNA", "short interfering RNA", "siRNA", "short interfering nucleic acid molecule", "short interfering oligonucleotide molecule", or "chemically-modified short interfering nucleic acid molecule" as used herein refers to any nucleic acid molecule capable of inhibiting or down regulating gene expression or viral replication, for example by mediating RNA interference "RNAi" or
25 gene silencing in a sequence-specific manner; see for example Bass, 2001, *Nature*, 411, 428-429; Elbashir *et al.*, 2001, *Nature*, 411, 494-498; and Kreutzer *et al.*, International PCT Publication No. WO 00/44895; Zernicka-Goetz *et al.*, International PCT Publication No. WO 01/36646; Fire, International PCT Publication No. WO 99/32619; Plaetinck *et al.*, International PCT Publication No. WO 00/01846; Mello and Fire,
30 International PCT Publication No. WO 01/29058; Deschamps-Depaillette, International PCT Publication No. WO 99/07409; and Li *et al.*, International PCT Publication No. WO

00/44914; Allshire, 2002, *Science*, 297, 1818-1819; Volpe *et al.*, 2002, *Science*, 297, 1833-1837; Jenuwein, 2002, *Science*, 297, 2215-2218; and Hall *et al.*, 2002, *Science*, 297, 2232-2237; Hutvagner and Zamore, 2002, *Science*, 297, 2056-60; McManus *et al.*, 2002, *RNA*, 8, 842-850; Reinhart *et al.*, 2002, *Gene & Dev.*, 16, 1616-1626; and Reinhart & Bartel, 2002, *Science*, 297, 1831). Non limiting examples of siNA molecules of the invention are shown in **Figures 4-6**, and **Tables II, III, and IV** herein. For example the siNA can be a double-stranded polynucleotide molecule comprising self-complementary sense and antisense regions, wherein the antisense region comprises nucleotide sequence that is complementary to nucleotide sequence in a target nucleic acid molecule or a portion thereof and the sense region having nucleotide sequence corresponding to the target nucleic acid sequence or a portion thereof. The siNA can be assembled from two separate oligonucleotides, where one strand is the sense strand and the other is the antisense strand, wherein the antisense and sense strands are self-complementary (i.e. each strand comprises nucleotide sequence that is complementary to nucleotide sequence in the other strand; such as where the antisense strand and sense strand form a duplex or double stranded structure, for example wherein the double stranded region is about 19 base pairs); the antisense strand comprises nucleotide sequence that is complementary to nucleotide sequence in a target nucleic acid molecule or a portion thereof and the sense strand comprises nucleotide sequence corresponding to the target nucleic acid sequence or a portion thereof. Alternatively, the siNA is assembled from a single oligonucleotide, where the self-complementary sense and antisense regions of the siNA are linked by means of a nucleic acid based or non-nucleic acid-based linker(s). The siNA can be a polynucleotide with a hairpin secondary structure, having self-complementary sense and antisense regions, wherein the antisense region comprises nucleotide sequence that is complementary to nucleotide sequence in a separate target nucleic acid molecule or a portion thereof and the sense region having nucleotide sequence corresponding to the target nucleic acid sequence or a portion thereof. The siNA can be a circular single-stranded polynucleotide having two or more loop structures and a stem comprising self-complementary sense and antisense regions, wherein the antisense region comprises nucleotide sequence that is complementary to nucleotide sequence in a target nucleic acid molecule or a portion thereof and the sense region having nucleotide sequence corresponding to the target nucleic acid sequence or a portion thereof, and wherein the circular polynucleotide can be processed either *in vivo* or *in vitro* to generate an active

siNA molecule capable of mediating RNAi. The siNA can also comprise a single stranded polynucleotide having nucleotide sequence complementary to nucleotide sequence in a target nucleic acid molecule or a portion thereof (for example, where such siNA molecule does not require the presence within the siNA molecule of nucleotide sequence corresponding to the target nucleic acid sequence or a portion thereof), wherein the single stranded polynucleotide can further comprise a terminal phosphate group, such as a 5'-phosphate (see for example Martinez *et al.*, 2002, *Cell*, 110, 563-574 and Schwarz *et al.*, 2002, *Molecular Cell*, 10, 537-568), or 5',3'-diphosphate. In certain embodiment, the siNA molecule of the invention comprises separate sense and antisense sequences or regions, wherein the sense and antisense regions are covalently linked by nucleotide or non-nucleotide linkers molecules as is known in the art, or are alternately non-covalently linked by ionic interactions, hydrogen bonding, van der waals interactions, hydrophobic interactions, and/or stacking interactions. In certain embodiments, the siNA molecules of the invention comprise nucleotide sequence that is complementary to nucleotide sequence of a target gene. In another embodiment, the siNA molecule of the invention interacts with nucleotide sequence of a target gene in a manner that causes inhibition of expression of the target gene. As used herein, siNA molecules need not be limited to those molecules containing only RNA, but further encompasses chemically-modified nucleotides and non-nucleotides. In certain embodiments, the short interfering nucleic acid molecules of the invention lack 2'-hydroxy (2'-OH) containing nucleotides. Applicant describes in certain embodiments short interfering nucleic acids that do not require the presence of nucleotides having a 2'-hydroxy group for mediating RNAi and as such, short interfering nucleic acid molecules of the invention optionally do not include any ribonucleotides (e.g., nucleotides having a 2'-OH group). Such siNA molecules that do not require the presence of ribonucleotides within the siNA molecule to support RNAi can however have an attached linker or linkers or other attached or associated groups, moieties, or chains containing one or more nucleotides with 2'-OH groups. Optionally, siNA molecules can comprise ribonucleotides at about 5, 10, 20, 30, 40, or 50% of the nucleotide positions. The modified short interfering nucleic acid molecules of the invention can also be referred to as short interfering modified oligonucleotides "siMON." As used herein, the term siNA is meant to be equivalent to other terms used to describe nucleic acid molecules that are capable of mediating sequence specific RNAi, for example short interfering RNA

(siRNA), double-stranded RNA (dsRNA), micro-RNA (miRNA), short hairpin RNA (shRNA), short interfering oligonucleotide, short interfering nucleic acid, short interfering modified oligonucleotide, chemically-modified siRNA, post-transcriptional gene silencing RNA (ptgsRNA), and others. In addition, as used herein, the term RNAi
5 is meant to be equivalent to other terms used to describe sequence specific RNA interference, such as post transcriptional gene silencing, or epigenetics. For example, siNA molecules of the invention can be used to epigenetically silence genes at both the post-transcriptional level or the pre-transcriptional level. In a non-limiting example, epigenetic regulation of gene expression by siNA molecules of the invention can result
10 from siNA mediated modification of chromatin structure to alter gene expression (see, for example, Allshire, 2002, *Science*, 297, 1818-1819; Volpe *et al.*, 2002, *Science*, 297, 1833-1837; Jenuwein, 2002, *Science*, 297, 2215-2218; and Hall *et al.*, 2002, *Science*, 297, 2232-2237).

By "modulate" is meant that the expression of the gene, or level of RNA molecule
15 or equivalent RNA molecules encoding one or more proteins or protein subunits, or activity of one or more proteins or protein subunits is up regulated or down regulated, such that expression, level, or activity is greater than or less than that observed in the absence of the modulator. For example, the term "modulate" can mean "inhibit," but the use of the word "modulate" is not limited to this definition.

20 By "inhibit" it is meant that the activity of a gene expression product or level of RNAs or equivalent RNAs encoding one or more gene products is reduced below that observed in the absence of the nucleic acid molecule of the invention. In one embodiment, inhibition with a siNA molecule preferably is below that level observed in the presence of an inactive or attenuated molecule that is unable to mediate an RNAi
25 response. In another embodiment, inhibition of gene expression with the siNA molecule of the instant invention is greater in the presence of the siNA molecule than in its absence.

By "inhibit", "down-regulate", or "reduce", it is meant that the expression of the gene, or level of RNA molecules or equivalent RNA molecules encoding one or more
30 proteins or protein subunits, or activity of one or more proteins or protein subunits, is reduced below that observed in the absence of the nucleic acid molecules (e.g., siNA) of

the invention. In one embodiment, inhibition, down-regulation or reduction with an siNA molecule is below that level observed in the presence of an inactive or attenuated molecule. In another embodiment, inhibition, down-regulation, or reduction with siNA molecules is below that level observed in the presence of, for example, an siNA molecule with scrambled sequence or with mismatches. In another embodiment, inhibition, down-regulation, or reduction of gene expression with a nucleic acid molecule of the instant invention is greater in the presence of the nucleic acid molecule than in its absence.

By "gene" or "target gene" is meant, a nucleic acid that encodes an RNA, for example, nucleic acid sequences including, but not limited to, structural genes encoding a polypeptide. The target gene can be a gene derived from a cell, an endogenous gene, a transgene, or exogenous genes such as genes of a pathogen, for example a virus, which is present in the cell after infection thereof. The cell containing the target gene can be derived from or contained in any organism, for example a plant, animal, protozoan, virus, bacterium, or fungus. Non-limiting examples of plants include monocots, dicots, or gymnosperms. Non-limiting examples of animals include vertebrates or invertebrates. Non-limiting examples of fungi include molds or yeasts.

By "HCV" as used herein is meant the hepatitis C Virus or any protein, peptide, or polypeptide, having hepatitis C virus activity or encoded by the HCV genome. The term "HCV" also includes nucleic acid molecules encoding RNA or protein(s) associated with the development and/or maintenance of HCV infection, such as nucleic acid molecules which encode HCV RNA or polypeptides (such as polynucleotides having Genbank Accession numbers shown in Table I), including polypeptides of different strains of HCV, mutant HCV genes, and splice variants of HCV genes, as well as genes involved in HCV pathways of gene expression and/or HCV activity. Also, the term "HCV" is meant to encompass HCV viral gene products and genes that modulate cellular targets for HCV infection, such as those described herein.

By "HCV protein" is meant, protein, peptide, or polypeptide, having hepatitis C virus activity or encoded by the HCV genome.

By "highly conserved sequence region" is meant, a nucleotide sequence of one or more regions in a target gene does not vary significantly from one generation to the other or from one biological system to the other.

By "sense region" is meant a nucleotide sequence of a siNA molecule having
5 complementarity to an antisense region of the siNA molecule. In addition, the sense region of a siNA molecule can comprise a nucleic acid sequence having homology with a target nucleic acid sequence.

By "antisense region" is meant a nucleotide sequence of a siNA molecule having complementarity to a target nucleic acid sequence. In addition, the antisense region of a
10 siNA molecule can optionally comprise a nucleic acid sequence having complementarity to a sense region of the siNA molecule.

By "target nucleic acid" is meant any nucleic acid sequence whose expression or activity is to be modulated. The target nucleic acid can be DNA or RNA.

By "complementarity" is meant that a nucleic acid can form hydrogen bond(s) with
15 another nucleic acid sequence by either traditional Watson-Crick or other non-traditional types. In reference to the nucleic molecules of the present invention, the binding free energy for a nucleic acid molecule with its complementary sequence is sufficient to allow the relevant function of the nucleic acid to proceed, e.g., RNAi activity. Determination of binding free energies for nucleic acid molecules is well known in the
20 art (see, e.g., Turner *et al.*, 1987, *CSH Symp. Quant. Biol.* LII pp.123-133; Frier *et al.*, 1986, *Proc. Nat. Acad. Sci. USA* 83:9373-9377; Turner *et al.*, 1987, *J. Am. Chem. Soc.* 109:3783-3785). A percent complementarity indicates the percentage of contiguous residues in a nucleic acid molecule that can form hydrogen bonds (e.g., Watson-Crick base pairing) with a second nucleic acid sequence (e.g., 5, 6, 7, 8, 9, 10 out of 10 being
25 50%, 60%, 70%, 80%, 90%, and 100% complementary). "Perfectly complementary" means that all the contiguous residues of a nucleic acid sequence will hydrogen bond with the same number of contiguous residues in a second nucleic acid sequence.

The siNA molecules of the invention represent a novel therapeutic approach to treat various diseases and conditions, including HCV infection, liver failure,

hepatocellular carcinoma, cirrhosis and any other indications that can respond to the level of HCV in a cell or tissue.

In one embodiment of the present invention, each sequence of a siNA molecule of the invention is independently about 18 to about 24 nucleotides in length, in specific
5 embodiments about 18, 19, 20, 21, 22, 23, or 24 nucleotides in length. In another embodiment, the siNA duplexes of the invention independently comprise about 17 to about 23 base pairs (*e.g.*, about 17, 18, 19, 20, 21, 22 or 23). In yet another embodiment, siNA molecules of the invention comprising hairpin or circular structures are about 35 to about 55 (*e.g.*, about 35, 40, 45, 50 or 55) nucleotides in length, or about 38 to about 44
10 (*e.g.*, 38, 39, 40, 41, 42, 43 or 44) nucleotides in length and comprising about 16 to about 22 (*e.g.*, about 16, 17, 18, 19, 20, 21 or 22) base pairs. Exemplary siNA molecules of the invention are shown in Table II. Exemplary synthetic siNA molecules of the invention are shown in Tables III and IV and/or Figures 4-5.

As used herein "cell" is used in its usual biological sense, and does not refer to an
15 entire multicellular organism, *e.g.*, specifically does not refer to a human. The cell can be present in an organism, *e.g.*, birds, plants and mammals such as humans, cows, sheep, apes, monkeys, swine, dogs, and cats. The cell can be prokaryotic (*e.g.*, bacterial cell) or eukaryotic (*e.g.*, mammalian or plant cell). The cell can be of somatic or germ line origin, totipotent or pluripotent, dividing or non-dividing. The cell can also be derived
20 from or can comprise a gamete or embryo, a stem cell, or a fully differentiated cell.

The siNA molecules of the invention are added directly, or can be complexed with cationic lipids, packaged within liposomes, or otherwise delivered to target cells or tissues. The nucleic acid or nucleic acid complexes can be locally administered to relevant tissues *ex vivo*, or *in vivo* through injection, infusion pump or stent, with or
25 without their incorporation in biopolymers. In particular embodiments, the nucleic acid molecules of the invention comprise sequences shown in Tables II-III and/or Figures 4-5. Examples of such nucleic acid molecules consist essentially of sequences defined in these tables and figures. Furthermore, the chemically modified constructs described in Table IV can be applied to any siNA sequence of the invention.

In another aspect, the invention provides mammalian cells containing one or more siNA molecules of this invention. The one or more siNA molecules can independently be targeted to the same or different sites.

By "RNA" is meant a molecule comprising at least one ribonucleotide residue. By
5 "ribonucleotide" is meant a nucleotide with a hydroxyl group at the 2' position of a β -D-ribo-furanose moiety. The terms include double-stranded RNA, single-stranded RNA, isolated RNA such as partially purified RNA, essentially pure RNA, synthetic RNA, recombinantly produced RNA, as well as altered RNA that differs from naturally occurring RNA by the addition, deletion, substitution and/or alteration of one or more
10 nucleotides. Such alterations can include addition of non-nucleotide material, such as to the end(s) of the siNA or internally, for example at one or more nucleotides of the RNA. Nucleotides in the RNA molecules of the instant invention can also comprise non-standard nucleotides, such as non-naturally occurring nucleotides or chemically synthesized nucleotides or deoxynucleotides. These altered RNAs can be referred to as
15 analogs or analogs of naturally-occurring RNA.

By "subject" is meant an organism, which is a donor or recipient of explanted cells or the cells themselves. "Subject" also refers to an organism to which the nucleic acid molecules of the invention can be administered. In one embodiment, a subject is a mammal or mammalian cells. In another embodiment, a subject is a human or human
20 cells.

The term "phosphorothioate" as used herein refers to an internucleotide linkage having Formula I, wherein Z and/or W comprise a sulfur atom. Hence, the term phosphorothioate refers to both phosphorothioate and phosphorodithioate internucleotide linkages.

25 The term "universal base" as used herein refers to nucleotide base analogs that form base pairs with each of the natural DNA/RNA bases with little discrimination between them. Non-limiting examples of universal bases include C-phenyl, C-naphthyl and other aromatic derivatives, inosine, azole carboxamides, and nitroazole derivatives such as 3-nitropyrrole, 4-nitroindole, 5-nitroindole, and 6-nitroindole as known in the art
30 (see for example Loakes, 2001, *Nucleic Acids Research*, 29, 2437-2447).

The term "acyclic nucleotide" as used herein refers to any nucleotide having an acyclic ribose sugar, for example where any of the ribose carbons (C1, C2, C3, C4, or C5), are independently or in combination absent from the nucleotide.

5 The nucleic acid molecules of the instant invention, individually, or in combination or in conjunction with other drugs, can be used to treat diseases or conditions discussed herein (e.g. A siRNA molecule of the invention can be adapted for use to treat for example HCV infection, liver failure, hepatocellular carcinoma, cirrhosis and other indications that can respond to the level of HCV in a cell or tissue). For example, to treat a particular disease or condition, the siNA molecules can be administered to a subject or
10 can be administered to other appropriate cells evident to those skilled in the art, individually or in combination with one or more drugs under conditions suitable for the treatment.

In a further embodiment, the siNA molecules can be used in combination with other known treatments to treat conditions or diseases discussed above. For example, the
15 described molecules could be used in combination with one or more known therapeutic agents to treat a disease or condition. Non-limiting examples of other therapeutic agents that can be readily combined with a siNA molecule of the invention are enzymatic nucleic acid molecules, allosteric nucleic acid molecules, antisense, decoy, or aptamer nucleic acid molecules, antibodies such as monoclonal antibodies, small molecules, and
20 other organic and/or inorganic compounds including metals, salts and ions.

In one embodiment, the invention features an expression vector comprising a nucleic acid sequence encoding at least one siNA molecule of the invention, in a manner which allows expression of the siNA molecule. For example, the vector can contain sequence(s) encoding both strands of a siNA molecule comprising a duplex. The vector
25 can also contain sequence(s) encoding a single nucleic acid molecule that is self-complementary and thus forms a siNA molecule. Non-limiting examples of such expression vectors are described in Paul *et al.*, 2002, *Nature Biotechnology*, 19, 505; Miyagishi and Taira, 2002, *Nature Biotechnology*, 19, 497; Lee *et al.*, 2002, *Nature Biotechnology*, 19, 500; and Novina *et al.*, 2002, *Nature Medicine*, advance online
30 publication doi:10.1038/nm725.

In another embodiment, the invention features a mammalian cell, for example, a human cell, including an expression vector of the invention.

In yet another embodiment, the expression vector of the invention comprises a sequence for a siNA molecule having complementarity to a RNA molecule referred to by a Genbank Accession numbers, for example Genbank Accession Nos. shown in Table I.

In one embodiment, an expression vector of the invention comprises a nucleic acid sequence encoding two or more siNA molecules, which can be the same or different.

In another aspect of the invention, siNA molecules that interact with target RNA molecules and down-regulate gene encoding target RNA molecules (for example target RNA molecules referred to by Genbank Accession numbers herein) are expressed from transcription units inserted into DNA or RNA vectors. The recombinant vectors can be DNA plasmids or viral vectors. siNA expressing viral vectors can be constructed based on, but not limited to, adeno-associated virus, retrovirus, adenovirus, or alphavirus. The recombinant vectors capable of expressing the siNA molecules can be delivered as described herein, and persist in target cells. Alternatively, viral vectors can be used that provide for transient expression of siNA molecules. Such vectors can be repeatedly administered as necessary. Once expressed, the siNA molecules bind and down-regulate gene function or expression via RNA interference (RNAi). Delivery of siNA expressing vectors can be systemic, such as by intravenous or intramuscular administration, by administration to target cells ex-planted from a subject followed by reintroduction into the subject, or by any other means that would allow for introduction into the desired target cell.

By "vectors" is meant any nucleic acid- and/or viral-based technique used to deliver a desired nucleic acid.

Other features and advantages of the invention will be apparent from the following description of the preferred embodiments thereof, and from the claims.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 shows a non-limiting example of a scheme for the synthesis of siNA molecules. The complementary siNA sequence strands, strand 1 and strand 2, are

synthesized in tandem and are connected by a cleavable linkage, such as a nucleotide succinate or abasic succinate, which can be the same or different from the cleavable linker used for solid phase synthesis on a solid support. The synthesis can be either solid phase or solution phase, in the example shown, the synthesis is a solid phase synthesis.

5 The synthesis is performed such that a protecting group, such as a dimethoxytrityl group, remains intact on the terminal nucleotide of the tandem oligonucleotide. Upon cleavage and deprotection of the oligonucleotide, the two siNA strands spontaneously hybridize to form a siNA duplex, which allows the purification of the duplex by utilizing the properties of the terminal protecting group, for example by applying a trityl on

10 purification method wherein only duplexes/oligonucleotides with the terminal protecting group are isolated.

Figure 2 shows a MALDI-TOV mass spectrum of a purified siNA duplex synthesized by a method of the invention. The two peaks shown correspond to the predicted mass of the separate siNA sequence strands. This result demonstrates that the

15 siNA duplex generated from tandem synthesis can be purified as a single entity using a simple trityl-on purification methodology.

Figure 3 shows a non-limiting proposed mechanistic representation of target RNA degradation involved in RNAi. Double-stranded RNA (dsRNA), which is generated by RNA-dependent RNA polymerase (RdRP) from foreign single-stranded RNA, for

20 example viral, transposon, or other exogenous RNA, activates the DICER enzyme that in turn generates siNA duplexes. Alternately, synthetic or expressed siNA can be introduced directly into a cell by appropriate means. An active siNA complex forms which recognizes a target RNA, resulting in degradation of the target RNA by the RISC endonuclease complex or in the synthesis of additional RNA by RNA-dependent RNA

25 polymerase (RdRP), which can activate DICER and result in additional siNA molecules, thereby amplifying the RNAi response.

Figure 4A-F shows non-limiting examples of chemically-modified siNA constructs of the present invention. In the figure, N stands for any nucleotide (adenosine, guanosine, cytosine, uridine, or optionally thymidine, for example thymidine can be

30 substituted in the overhanging regions designated by parenthesis (N N). Various modifications are shown for the sense and antisense strands of the siNA constructs.

Figure 4A: The sense strand comprises 21 nucleotides having four phosphorothioate 5'- and 3'-terminal internucleotide linkages, wherein the two terminal 3'-nucleotides are optionally base paired and wherein all pyrimidine nucleotides that may be present are 2'-O-methyl or 2'-deoxy-2'-fluoro modified nucleotides except for (N N) nucleotides, which can comprise ribonucleotides, deoxynucleotides, universal bases, or other chemical modifications described herein. The antisense strand comprises 21 nucleotides, optionally having a 3'-terminal glyceryl moiety and wherein the two terminal 3'-nucleotides are optionally complementary to the target RNA sequence, and having one 3'-terminal phosphorothioate internucleotide linkage and four 5'-terminal phosphorothioate internucleotide linkages and wherein all pyrimidine nucleotides that may be present are 2'-deoxy-2'-fluoro modified nucleotides except for (N N) nucleotides, which can comprise ribonucleotides, deoxynucleotides, universal bases, or other chemical modifications described herein.

Figure 4B: The sense strand comprises 21 nucleotides wherein the two terminal 3'-nucleotides are optionally base paired and wherein all pyrimidine nucleotides that may be present are 2'-O-methyl or 2'-deoxy-2'-fluoro modified nucleotides except for (N N) nucleotides, which can comprise ribonucleotides, deoxynucleotides, universal bases, or other chemical modifications described herein. The antisense strand comprises 21 nucleotides, optionally having a 3'-terminal glyceryl moiety and wherein the two terminal 3'-nucleotides are optionally complementary to the target RNA sequence, and wherein all pyrimidine nucleotides that may be present are 2'-deoxy-2'-fluoro modified nucleotides except for (N N) nucleotides, which can comprise ribonucleotides, deoxynucleotides, universal bases, or other chemical modifications described herein.

Figure 4C: The sense strand comprises 21 nucleotides having 5'- and 3'- terminal cap moieties wherein the two terminal 3'-nucleotides are optionally base paired and wherein all pyrimidine nucleotides that may be present are 2'-O-methyl or 2'-deoxy-2'-fluoro modified nucleotides except for (N N) nucleotides, which can comprise ribonucleotides, deoxynucleotides, universal bases, or other chemical modifications described herein. The antisense strand comprises 21 nucleotides, optionally having a 3'-terminal glyceryl moiety and wherein the two terminal 3'-nucleotides are optionally complementary to the target RNA sequence, and having one 3'-terminal phosphorothioate internucleotide linkage and wherein all pyrimidine nucleotides that

may be present are 2'-deoxy-2'-fluoro modified nucleotides except for (N N) nucleotides, which can comprise ribonucleotides, deoxynucleotides, universal bases, or other chemical modifications described herein.

Figure 4D: The sense strand comprises 21 nucleotides having 5'- and 3'- terminal cap moieties wherein the two terminal 3'-nucleotides are optionally base paired and wherein all pyrimidine nucleotides that may be present are 2'-deoxy-2'-fluoro modified nucleotides except for (N N) nucleotides, which can comprise ribonucleotides, deoxynucleotides, universal bases, or other chemical modifications described herein and wherein all purine nucleotides that may be present are 2'-deoxy nucleotides. The antisense strand comprises 21 nucleotides, optionally having a 3'-terminal glyceryl moiety and wherein the two terminal 3'-nucleotides are optionally complementary to the target RNA sequence, and having one 3'-terminal phosphorothioate internucleotide linkage and wherein all pyrimidine nucleotides that may be present are 2'-deoxy-2'-fluoro modified nucleotides and all purine nucleotides that may be present are 2'-O-methyl modified nucleotides except for (N N) nucleotides, which can comprise ribonucleotides, deoxynucleotides, universal bases, or other chemical modifications described herein.

Figure 4E: The sense strand comprises 21 nucleotides having 5'- and 3'- terminal cap moieties wherein the two terminal 3'-nucleotides are optionally base paired and wherein all pyrimidine nucleotides that may be present are 2'-deoxy-2'-fluoro modified nucleotides except for (N N) nucleotides, which can comprise ribonucleotides, deoxynucleotides, universal bases, or other chemical modifications described herein. The antisense strand comprises 21 nucleotides, optionally having a 3'-terminal glyceryl moiety and wherein the two terminal 3'-nucleotides are optionally complementary to the target RNA sequence, and wherein all pyrimidine nucleotides that may be present are 2'-deoxy-2'-fluoro modified nucleotides and all purine nucleotides that may be present are 2'-O-methyl modified nucleotides except for (N N) nucleotides, which can comprise ribonucleotides, deoxynucleotides, universal bases, or other chemical modifications described herein.

Figure 4F: The sense strand comprises 21 nucleotides having 5'- and 3'- terminal cap moieties wherein the two terminal 3'-nucleotides are optionally base paired and wherein all pyrimidine nucleotides that may be present are 2'-deoxy-2'-fluoro modified

nucleotides except for (N N) nucleotides, which can comprise ribonucleotides, deoxynucleotides, universal bases, or other chemical modifications described herein. The antisense strand comprises 21 nucleotides, optionally having a 3'-terminal glyceryl moiety and wherein the two terminal 3'-nucleotides are optionally complementary to the target RNA sequence, and having one 3'-terminal phosphorothioate internucleotide linkage and wherein all pyrimidine nucleotides that may be present are 2'-deoxy-2'-fluoro modified nucleotides and all purine nucleotides that may be present are 2'-deoxy nucleotides except for (N N) nucleotides, which can comprise ribonucleotides, deoxynucleotides, universal bases, or other chemical modifications described herein.

5 The antisense strand of constructs A-F comprise sequence complementary to any target nucleic acid sequence of the invention.

10

Figure 5A-F shows non-limiting examples of specific chemically-modified siNA sequences of the invention. A-F applies the chemical modifications described in Figure 4A-F to a HCV siNA sequence.

15 Figure 6 shows non-limiting examples of different siNA constructs of the invention. The examples shown (constructs 1, 2, and 3) have 19 representative base pairs; however, different embodiments of the invention include any number of base pairs described herein. Bracketed regions represent nucleotide overhangs, for example comprising about 1, 2, 3, or 4 nucleotides in length, preferably about 2 nucleotides.

20 Constructs 1 and 2 can be used independently for RNAi activity. Construct 2 can comprise a polynucleotide or non-nucleotide linker, which can optionally be designed as a biodegradable linker. In one embodiment, the loop structure shown in construct 2 can comprise a biodegradable linker that results in the formation of construct 1 *in vivo* and/or *in vitro*. In another example, construct 3 can be used to generate construct 2 under the same principle wherein a linker is used to generate the active siNA construct 2 *in vivo* and/or *in vitro*, which can optionally utilize another biodegradable linker to generate the active siNA construct 1 *in vivo* and/or *in vitro*. As such, the stability and/or activity of the siNA constructs can be modulated based on the design of the siNA construct for use *in vivo* or *in vitro* and/or *in vitro*.

25

30 Figure 7A-C is a diagrammatic representation of a scheme utilized in generating an expression cassette to generate siNA hairpin constructs.

Figure 7A: A DNA oligomer is synthesized with a 5'-restriction site (R1) sequence followed by a region having sequence identical (sense region of siNA) to a predetermined HCV target sequence, wherein the sense region comprises, for example, about 19, 20, 21, or 22 nucleotides (N) in length, which is followed by a loop sequence
5 of defined sequence (X), comprising, for example, about 3 to about 10 nucleotides.

Figure 7B: The synthetic construct is then extended by DNA polymerase to generate a hairpin structure having self-complementary sequence that will result in a siNA transcript having specificity for a HCV target sequence and having self-complementary sense and antisense regions.

10 **Figure 7C:** The construct is heated (for example to about 95°C) to linearize the sequence, thus allowing extension of a complementary second DNA strand using a primer to the 3'-restriction sequence of the first strand. The double-stranded DNA is then inserted into an appropriate vector for expression in cells. The construct can be designed such that a 3'-terminal nucleotide overhang results from the transcription, for example by
15 engineering restriction sites and/or utilizing a poly-U termination region as described in Paul *et al.*, 2002, *Nature Biotechnology*, 29, 505-508.

Figure 8A-C is a diagrammatic representation of a scheme utilized in generating an expression cassette to generate double-stranded siNA constructs.

Figure 8A: A DNA oligomer is synthesized with a 5'-restriction (R1) site sequence
20 followed by a region having sequence identical (sense region of siNA) to a predetermined HCV target sequence, wherein the sense region comprises, for example, about 19, 20, 21, or 22 nucleotides (N) in length, and which is followed by a 3'-restriction site (R2) which is adjacent to a loop sequence of defined sequence (X).

Figure 8B: The synthetic construct is then extended by DNA polymerase to
25 generate a hairpin structure having self-complementary sequence.

Figure 8C: The construct is processed by restriction enzymes specific to R1 and R2 to generate a double-stranded DNA which is then inserted into an appropriate vector for expression in cells. The transcription cassette is designed such that a U6 promoter region flanks each side of the dsDNA which generates the separate sense and antisense

strands of the siNA. Poly T termination sequences can be added to the constructs to generate U overhangs in the resulting transcript.

Figure 9A-E is a diagrammatic representation of a method used to determine target sites for siNA mediated RNAi within a particular target nucleic acid sequence, such as messenger RNA.

Figure 9A: A pool of siNA oligonucleotides are synthesized wherein the antisense region of the siNA constructs has complementarity to target sites across the target nucleic acid sequence, and wherein the sense region comprises sequence complementary to the antisense region of the siNA.

Figure 9B&C: (Figure 9B) The sequences are pooled and are inserted into vectors such that (Figure 9C) transfection of a vector into cells results in the expression of the siNA.

Figure 9D: Cells are sorted based on phenotypic change that is associated with modulation of the target nucleic acid sequence.

Figure 9E: The siNA is isolated from the sorted cells and is sequenced to identify efficacious target sites within the target nucleic acid sequence.

Figure 10 shows non-limiting examples of different stabilization chemistries (1-10) that can be used, for example, to stabilize the 3'-end of siNA sequences of the invention, including (1) [3'-3']-inverted deoxyribose; (2) deoxyribonucleotide; (3) [5'-3']-3'-deoxyribonucleotide; (4) [5'-3']-ribonucleotide; (5) [5'-3']-3'-O-methyl ribonucleotide; (6) 3'-glyceryl; (7) [3'-5']-3'-deoxyribonucleotide; (8) [3'-3']-deoxyribonucleotide; (9) [5'-2']-deoxyribonucleotide; and (10) [5-3']-dideoxyribonucleotide. In addition to modified and unmodified backbone chemistries indicated in the figure, these chemistries can be combined with different backbone modifications as described herein, for example, backbone modifications having Formula I. In addition, the 2'-deoxy nucleotide shown 5' to the terminal modifications shown can be another modified or unmodified nucleotide or non-nucleotide described herein, for example modifications having any of Formulae I-VII or any combination thereof.

Figure 11 shows a non-limiting example of a strategy used to identify chemically modified siNA constructs of the invention that are nuclease resistance while preserving the ability to mediate RNAi activity. Chemical modifications are introduced into the siNA construct based on educated design parameters (e.g. introducing 2'-mofications, base modifications, backbone modifications, terminal cap modifications etc). The modified construct is tested in an appropriate system (e.g. human serum for nuclease resistance, shown, or an animal model for PK/delivery parameters). In parallel, the siNA construct is tested for RNAi activity, for example in a cell culture system such as a luciferase reporter assay). Lead siNA constructs are then identified which possess a particular characteristic while maintaining RNAi activity, and can be further modified and assayed once again. This same approach can be used to identify siNA-conjugate molecules with improved pharmacokinetic profiles, delivery, and RNAi activity.

Figure 12 shows a non-limiting example of siRNA constructs 29579/ 29586 and 29578/ 29585 targeting viral replication of an HCV/poliovirus chimera in comparison to an inverse siNA control construct 29593/29600.

Figure 13 shows a non-limiting example of a dose response study of a siRNA construct 29579/ 29586 targeting viral replication of an HCV/poliovirus chimera in comparison to an inverse siNA control construct 29593/ 29600. The inhibition of HCV/poliovirus chimera replication by 29579/ 29586 siNA construct was measured at 1 nM, 5 nM, 10 nM, and 25 nM concentrations of 29579/ 29586 siNA construct.

Figure 14 shows a non-limiting example of a chemically modified siRNA construct 30051/30053 targeting viral replication of an HCV/poliovirus chimera in comparison to an inverse siNA control construct 30052/ 30054.

Figure 15 shows a non-limiting example of a chemically modified siRNA construct 30055/ 30057 targeting viral replication of an HCV/poliovirus chimera in comparison to an inverse siNA control construct 30056/ 30058.

Figure 16 shows a non-limiting example of several chemically modified siRNA constructs targeting viral replication of an HCV/poliovirus chimera at 10 nM treatment in comparison to a lipid control and an inverse siNA control construct 29593/ 29600.

Figure 17 shows a non-limiting example of several chemically modified siRNA constructs targeting viral replication of a HCV/poliovirus chimera at 25 nM treatment in comparison to a lipid control and an inverse siNA control construct 29593/ 29600.

5 Figure 18 shows a non-limiting example of several chemically modified siRNA constructs targeting viral replication of a Huh7 HCV replicon system at 25 nM treatment in comparison to untreated cells ("cells"), cells transfected with lipofectamine ("LFA2K") and inverse siNA control constructs.

DETAILED DESCRIPTION OF THE INVENTION

Mechanism of action of Nucleic Acid Molecules of the Invention

10 The discussion that follows discusses the proposed mechanism of RNA interference mediated by short interfering RNA as is presently known, and is not meant to be limiting and is not an admission of prior art. Applicant demonstrates herein that chemically-modified short interfering nucleic acids possess similar or improved capacity to mediate RNAi as do siRNA molecules and are expected to possess improved stability
15 and activity *in vivo*; therefore, this discussion is not meant to be limiting only to siRNA and can be applied to siNA as a whole. By "improved capacity to mediate RNAi" or "improved RNAi activity" is meant to include RNAi activity measured *in vitro* and/or *in vivo* where the RNAi activity is a reflection of both the ability of the siNA to mediate RNAi and the stability of the siNAs of the invention. In this invention, the product of
20 these activities can be increased *in vitro* and/or *in vivo* compared to an all RNA siRNA or a siNA containing a plurality of ribonucleotides. In some cases, the activity or stability of the siNA molecule can be decreased (i.e., less than ten-fold), but the overall activity of the siNA molecule is enhanced *in vitro* and/or *in vivo*.

RNA interference refers to the process of sequence specific post-transcriptional
25 gene silencing in animals mediated by short interfering RNAs (siRNAs) (Fire *et al.*, 1998, *Nature*, 391, 806). The corresponding process in plants is commonly referred to as post-transcriptional gene silencing or RNA silencing and is also referred to as quelling in fungi. The process of post-transcriptional gene silencing is thought to be an evolutionarily-conserved cellular defense mechanism used to prevent the expression of
30 foreign genes which is commonly shared by diverse flora and phyla (Fire *et al.*, 1999,

Trends Genet., 15, 358). Such protection from foreign gene expression may have evolved in response to the production of double-stranded RNAs (dsRNAs) derived from viral infection or the random integration of transposon elements into a host genome via a cellular response that specifically destroys homologous single-stranded RNA or viral genomic RNA. The presence of dsRNA in cells triggers the RNAi response through a mechanism that has yet to be fully characterized. This mechanism appears to be different from the interferon response that results from dsRNA-mediated activation of protein kinase PKR and 2', 5'-oligoadenylate synthetase resulting in non-specific cleavage of mRNA by ribonuclease L.

10 The presence of long dsRNAs in cells stimulates the activity of a ribonuclease III enzyme referred to as Dicer. Dicer is involved in the processing of the dsRNA into short pieces of dsRNA known as short interfering RNAs (siRNAs) (Berstein *et al.*, 2001, *Nature*, 409, 363). Short interfering RNAs derived from Dicer activity are typically about 21 to about 23 nucleotides in length and comprise about 19 base pair duplexes.

15 Dicer has also been implicated in the excision of 21- and 22-nucleotide small temporal RNAs (stRNAs) from precursor RNA of conserved structure that are implicated in translational control (Hutvagner *et al.*, 2001, *Science*, 293, 834). The RNAi response also features an endonuclease complex containing a siRNA, commonly referred to as an RNA-induced silencing complex (RISC), which mediates cleavage of single-stranded

20 RNA having sequence homologous to the siRNA. Cleavage of the target RNA takes place in the middle of the region complementary to the guide sequence of the siRNA duplex (Elbashir *et al.*, 2001, *Genes Dev.*, 15, 188). In addition, RNA interference can also involve small RNA (e.g., micro-RNA or miRNA) mediated gene silencing, presumably through cellular mechanisms that regulate chromatin structure and thereby

25 prevent transcription of target gene sequences (see for example Allshire, 2002, *Science*, 297, 1818-1819; Volpe *et al.*, 2002, *Science*, 297, 1833-1837; Jenuwein, 2002, *Science*, 297, 2215-2218; and Hall *et al.*, 2002, *Science*, 297, 2232-2237). As such, siRNA molecules of the invention can be used to mediate gene silencing via interaction with RNA transcripts or alternately by interaction with particular gene sequences, wherein

30 such interaction results in gene silencing either at the transcriptional level or post-transcriptional level.

RNAi has been studied in a variety of systems. Fire *et al.*, 1998, *Nature*, 391, 806, were the first to observe RNAi in *C. elegans*. Wianny and Goetz, 1999, *Nature Cell Biol.*, 2, 70, describe RNAi mediated by dsRNA in mouse embryos. Hammond *et al.*, 2000, *Nature*, 404, 293, describe RNAi in *Drosophila* cells transfected with dsRNA. 5 Elbashir *et al.*, 2001, *Nature*, 411, 494, describe RNAi induced by introduction of duplexes of synthetic 21-nucleotide RNAs in cultured mammalian cells including human embryonic kidney and HeLa cells. Recent work in *Drosophila* embryonic lysates has revealed certain requirements for siRNA length, structure, chemical composition, and sequence that are essential to mediate efficient RNAi activity. These studies have shown 10 that 21 nucleotide siRNA duplexes are most active when containing two 2-nucleotide 3'-terminal nucleotide overhangs. Furthermore, substitution of one or both siRNA strands with 2'-deoxy or 2'-O-methyl nucleotides abolishes RNAi activity, whereas substitution of 3'-terminal siRNA nucleotides with deoxy nucleotides was shown to be tolerated. Mismatch sequences in the center of the siRNA duplex were also shown to abolish RNAi 15 activity. In addition, these studies also indicate that the position of the cleavage site in the target RNA is defined by the 5'-end of the siRNA guide sequence rather than the 3'-end (Elbashir *et al.*, 2001, *EMBO J.*, 20, 6877). Other studies have indicated that a 5'-phosphate on the target-complementary strand of a siRNA duplex is required for siRNA activity and that ATP is utilized to maintain the 5'-phosphate moiety on the siRNA 20 (Nykanen *et al.*, 2001, *Cell*, 107, 309); however, siRNA molecules lacking a 5'-phosphate are active when introduced exogenously, suggesting that 5'-phosphorylation of siRNA constructs may occur *in vivo*.

Synthesis of Nucleic acid Molecules

Synthesis of nucleic acids greater than 100 nucleotides in length is difficult using 25 automated methods, and the therapeutic cost of such molecules is prohibitive. In this invention, small nucleic acid motifs ("small" refers to nucleic acid motifs no more than 100 nucleotides in length, preferably no more than 80 nucleotides in length, and most preferably no more than 50 nucleotides in length; *e.g.*, individual siNA oligonucleotide sequences or siNA sequences synthesized in tandem) are preferably used for exogenous 30 delivery. The simple structure of these molecules increases the ability of the nucleic acid to invade targeted regions of protein and/or RNA structure. Exemplary molecules of the instant invention are chemically synthesized, and others can similarly be synthesized.

Oligonucleotides (*e.g.*, certain modified oligonucleotides or portions of oligonucleotides lacking ribonucleotides) are synthesized using protocols known in the art, for example as described in Caruthers *et al.*, 1992, *Methods in Enzymology* 211, 3-19, Thompson *et al.*, International PCT Publication No. WO 99/54459, Wincott *et al.*, 5 1995, *Nucleic Acids Res.* 23, 2677-2684, Wincott *et al.*, 1997, *Methods Mol. Bio.*, 74, 59, Brennan *et al.*, 1998, *Biotechnol Bioeng.*, 61, 33-45, and Brennan, U.S. Pat. No. 6,001,311. All of these references are incorporated herein by reference. The synthesis of oligonucleotides makes use of common nucleic acid protecting and coupling groups, such as dimethoxytrityl at the 5'-end, and phosphoramidites at the 3'-end. In a non-10 limiting example, small scale syntheses are conducted on a 394 Applied Biosystems, Inc. synthesizer using a 0.2 μ mol scale protocol with a 2.5 min coupling step for 2'-O-methylated nucleotides and a 45 sec coupling step for 2'-deoxy nucleotides or 2'-deoxy-2'-fluoro nucleotides. Table V outlines the amounts and the contact times of the reagents used in the synthesis cycle. Alternatively, syntheses at the 0.2 μ mol scale can be 15 performed on a 96-well plate synthesizer, such as the instrument produced by Protogene (Palo Alto, CA) with minimal modification to the cycle. A 33-fold excess (60 μ L of 0.11 M = 6.6 μ mol) of 2'-O-methyl phosphoramidite and a 105-fold excess of S-ethyl tetrazole (60 μ L of 0.25 M = 15 μ mol) can be used in each coupling cycle of 2'-O-methyl residues relative to polymer-bound 5'-hydroxyl. A 22-fold excess (40 μ L of 0.11 M = 20 4.4 μ mol) of deoxy phosphoramidite and a 70-fold excess of S-ethyl tetrazole (40 μ L of 0.25 M = 10 μ mol) can be used in each coupling cycle of deoxy residues relative to polymer-bound 5'-hydroxyl. Average coupling yields on the 394 Applied Biosystems, Inc. synthesizer, determined by colorimetric quantitation of the trityl fractions, are typically 97.5-99%. Other oligonucleotide synthesis reagents for the 394 Applied 25 Biosystems, Inc. synthesizer include the following: detritylation solution is 3% TCA in methylene chloride (ABI); capping is performed with 16% *N*-methyl imidazole in THF (ABI) and 10% acetic anhydride/10% 2,6-lutidine in THF (ABI); and oxidation solution is 16.9 mM I₂, 49 mM pyridine, 9% water in THF (PERSEPTIVE™). Burdick & Jackson Synthesis Grade acetonitrile is used directly from the reagent bottle. S-30 Ethyltetrazole solution (0.25 M in acetonitrile) is made up from the solid obtained from American International Chemical, Inc. Alternately, for the introduction of phosphorothioate linkages, Beaucage reagent (3H-1,2-Benzodithiol-3-one 1,1-dioxide, 0.05 M in acetonitrile) is used.

Deprotection of the DNA-based oligonucleotides is performed as follows: the polymer-bound trityl-on oligoribonucleotide is transferred to a 4 mL glass screw top vial and suspended in a solution of 40% aq. methylamine (1 mL) at 65 °C for 10 min. After cooling to -20 °C, the supernatant is removed from the polymer support. The support is
5 washed three times with 1.0 mL of EtOH:MeCN:H₂O/3:1:1, vortexed and the supernatant is then added to the first supernatant. The combined supernatants, containing the oligoribonucleotide, are dried to a white powder.

The method of synthesis used for RNA including certain siNA molecules of the invention follows the procedure as described in Usman *et al.*, 1987, *J. Am. Chem. Soc.*,
10 109, 7845; Scaringe *et al.*, 1990, *Nucleic Acids Res.*, 18, 5433; and Wincott *et al.*, 1995, *Nucleic Acids Res.* 23, 2677-2684 Wincott *et al.*, 1997, *Methods Mol. Bio.*, 74, 59, and makes use of common nucleic acid protecting and coupling groups, such as dimethoxytrityl at the 5'-end, and phosphoramidites at the 3'-end. In a non-limiting example, small scale syntheses are conducted on a 394 Applied Biosystems, Inc.
15 synthesizer using a 0.2 µmol scale protocol with a 7.5 min coupling step for alkylsilyl protected nucleotides and a 2.5 min coupling step for 2'-O-methylated nucleotides. Table V outlines the amounts and the contact times of the reagents used in the synthesis cycle. Alternatively, syntheses at the 0.2 µmol scale can be done on a 96-well plate synthesizer, such as the instrument produced by Protogene (Palo Alto, CA) with minimal
20 modification to the cycle. A 33-fold excess (60 µL of 0.11 M = 6.6 µmol) of 2'-O-methyl phosphoramidite and a 75-fold excess of S-ethyl tetrazole (60 µL of 0.25 M = 15 µmol) can be used in each coupling cycle of 2'-O-methyl residues relative to polymer-bound 5'-hydroxyl. A 66-fold excess (120 µL of 0.11 M = 13.2 µmol) of alkylsilyl (ribo) protected phosphoramidite and a 150-fold excess of S-ethyl tetrazole (120 µL of 0.25 M
25 = 30 µmol) can be used in each coupling cycle of ribo residues relative to polymer-bound 5'-hydroxyl. Average coupling yields on the 394 Applied Biosystems, Inc. synthesizer, determined by colorimetric quantitation of the trityl fractions, are typically 97.5-99%. Other oligonucleotide synthesis reagents for the 394 Applied Biosystems, Inc. synthesizer include the following: detritylation solution is 3% TCA in methylene
30 chloride (ABI); capping is performed with 16% *N*-methyl imidazole in THF (ABI) and 10% acetic anhydride/10% 2,6-lutidine in THF (ABI); oxidation solution is 16.9 mM I₂, 49 mM pyridine, 9% water in THF (PERSEPTIVE™). Burdick & Jackson Synthesis

Grade acetonitrile is used directly from the reagent bottle. S-Ethyltetrazole solution (0.25 M in acetonitrile) is made up from the solid obtained from American International Chemical, Inc. Alternately, for the introduction of phosphorothioate linkages, Beaucage reagent (3H-1,2-Benzodithiol-3-one 1,1-dioxide 0.05 M in acetonitrile) is used.

5 Deprotection of the RNA is performed using either a two-pot or one-pot protocol. For the two-pot protocol, the polymer-bound trityl-on oligoribonucleotide is transferred to a 4 mL glass screw top vial and suspended in a solution of 40% aq. methylamine (1 mL) at 65 °C for 10 min. After cooling to -20 °C, the supernatant is removed from the polymer support. The support is washed three times with 1.0 mL of
10 EtOH:MeCN:H₂O/3:1:1, vortexed and the supernatant is then added to the first supernatant. The combined supernatants, containing the oligoribonucleotide, are dried to a white powder. The base deprotected oligoribonucleotide is resuspended in anhydrous TEA/HF/NMP solution (300 µL of a solution of 1.5 mL N-methylpyrrolidinone, 750 µL TEA and 1 mL TEA•3HF to provide a 1.4 M HF concentration) and heated to 65 °C.
15 After 1.5 h, the oligomer is quenched with 1.5 M NH₄HCO₃.

Alternatively, for the one-pot protocol, the polymer-bound trityl-on oligoribonucleotide is transferred to a 4 mL glass screw top vial and suspended in a solution of 33% ethanolic methylamine/DMSO: 1/1 (0.8 mL) at 65 °C for 15 min. The vial is brought to rt. TEA•3HF (0.1 mL) is added and the vial is heated at 65 °C for 15
20 min. The sample is cooled at -20 °C and then quenched with 1.5 M NH₄HCO₃.

For purification of the trityl-on oligomers, the quenched NH₄HCO₃ solution is loaded onto a C-18 containing cartridge that had been prewashed with acetonitrile followed by 50 mM TEAA. After washing the loaded cartridge with water, the RNA is detritylated with 0.5% TFA for 13 min. The cartridge is then washed again with water,
25 salt exchanged with 1 M NaCl and washed with water again. The oligonucleotide is then eluted with 30% acetonitrile.

The average stepwise coupling yields are typically >98% (Wincott *et al.*, 1995 *Nucleic Acids Res.* 23, 2677-2684). Those of ordinary skill in the art will recognize that the scale of synthesis can be adapted to be larger or smaller than the example described
30 above including but not limited to 96-well format.

Alternatively, the nucleic acid molecules of the present invention can be synthesized separately and joined together post-synthetically, for example, by ligation (Moore *et al.*, 1992, *Science* 256, 9923; Draper *et al.*, International PCT publication No. WO 93/23569; Shabarova *et al.*, 1991, *Nucleic Acids Research* 19, 4247; Bellon *et al.*, 5 1997, *Nucleosides & Nucleotides*, 16, 951; Bellon *et al.*, 1997, *Bioconjugate Chem.* 8, 204), or by hybridization following synthesis and/or deprotection.

The siNA molecules of the invention can also be synthesized via a tandem synthesis methodology as described in Example 1 herein, wherein both siNA strands are synthesized as a single contiguous oligonucleotide fragment or strand separated by a 10 cleavable linker which is subsequently cleaved to provide separate siNA fragments or strands that hybridize and permit purification of the siNA duplex. The linker can be a polynucleotide linker or a non-nucleotide linker. The tandem synthesis of siNA as described herein can be readily adapted to both multiwell/multiplate synthesis platforms such as 96 well or similarly larger multi-well platforms. The tandem synthesis of siNA as 15 described herein can also be readily adapted to large scale synthesis platforms employing batch reactors, synthesis columns and the like.

A siNA molecule can also be assembled from two distinct nucleic acid strands or fragments wherein one fragment includes the sense region and the second fragment includes the antisense region of the RNA molecule.

20 The nucleic acid molecules of the present invention can be modified extensively to enhance stability by modification with nuclease resistant groups, for example, 2'-amino, 2'-C-allyl, 2'-fluoro, 2'-O-methyl, 2'-H (for a review see Usman and Cedergren, 1992, *TIBS* 17, 34; Usman *et al.*, 1994, *Nucleic Acids Symp. Ser.* 31, 163). siNA constructs can be purified by gel electrophoresis using general methods or can be purified by high 25 pressure liquid chromatography (HPLC; see Wincott *et al.*, *supra*, the totality of which is hereby incorporated herein by reference) and re-suspended in water.

In another aspect of the invention, siNA molecules of the invention are expressed from transcription units inserted into DNA or RNA vectors. The recombinant vectors can be DNA plasmids or viral vectors. siNA expressing viral vectors can be constructed 30 based on, but not limited to, adeno-associated virus, retrovirus, adenovirus, or alphavirus. The recombinant vectors capable of expressing the siNA molecules can be delivered as

described herein, and persist in target cells. Alternatively, viral vectors can be used that provide for transient expression of siNA molecules.

Optimizing Activity of the nucleic acid molecule of the invention.

Chemically synthesizing nucleic acid molecules with modifications (base, sugar
5 and/or phosphate) can prevent their degradation by serum ribonucleases, which can increase their potency (see *e.g.*, Eckstein *et al.*, International Publication No. WO 92/07065; Perrault *et al.*, 1990 *Nature* 344, 565; Pieken *et al.*, 1991, *Science* 253, 314; Usman and Cedergren, 1992, *Trends in Biochem. Sci.* 17, 334; Usman *et al.*, International Publication No. WO 93/15187; and Rossi *et al.*, International Publication
10 No. WO 91/03162; Sproat, U.S. Pat. No. 5,334,711; Gold *et al.*, U.S. Pat. No. 6,300,074; and Burgin *et al.*, *supra*; all of which are incorporated by reference herein). All of the above references describe various chemical modifications that can be made to the base, phosphate and/or sugar moieties of the nucleic acid molecules described herein. Modifications that enhance their efficacy in cells, and removal of bases from nucleic acid
15 molecules to shorten oligonucleotide synthesis times and reduce chemical requirements are desired.

There are several examples in the art describing sugar, base and phosphate modifications that can be introduced into nucleic acid molecules with significant enhancement in their nuclease stability and efficacy. For example, oligonucleotides are
20 modified to enhance stability and/or enhance biological activity by modification with nuclease resistant groups, for example, 2'-amino, 2'-C-allyl, 2'-fluoro, 2'-O-methyl, 2'-O-allyl, 2'-H, nucleotide base modifications (for a review see Usman and Cedergren, 1992, *TIBS*. 17, 34; Usman *et al.*, 1994, *Nucleic Acids Symp. Ser.* 31, 163; Burgin *et al.*, 1996, *Biochemistry*, 35, 14090). Sugar modification of nucleic acid molecules have been
25 extensively described in the art (see Eckstein *et al.*, International Publication PCT No. WO 92/07065; Perrault *et al.* *Nature*, 1990, 344, 565-568; Pieken *et al.* *Science*, 1991, 253, 314-317; Usman and Cedergren, *Trends in Biochem. Sci.* , 1992, 17, 334-339; Usman *et al.* International Publication PCT No. WO 93/15187; Sproat, U.S. Pat. No. 5,334,711 and Beigelman *et al.*, 1995, *J. Biol. Chem.*, 270, 25702; Beigelman *et al.*,
30 International PCT publication No. WO 97/26270; Beigelman *et al.*, U.S. Pat. No. 5,716,824; Usman *et al.*, U.S. Pat. No. 5,627,053; Woolf *et al.*, International PCT

Publication No. WO 98/13526; Thompson *et al.*, USSN 60/082,404 which was filed on April 20, 1998; Karpeisky *et al.*, 1998, *Tetrahedron Lett.*, 39, 1131; Earnshaw and Gait, 1998, *Biopolymers (Nucleic Acid Sciences)*, 48, 39-55; Verma and Eckstein, 1998, *Annu. Rev. Biochem.*, 67, 99-134; and Burlina *et al.*, 1997, *Bioorg. Med. Chem.*, 5, 1999-2010; all of the references are hereby incorporated in their totality by reference herein). Such publications describe general methods and strategies to determine the location of incorporation of sugar, base and/or phosphate modifications and the like into nucleic acid molecules without modulating catalysis, and are incorporated by reference herein. In view of such teachings, similar modifications can be used as described herein to modify the siNA nucleic acid molecules of the instant invention so long as the ability of siNA to promote RNAi in cells is not significantly inhibited.

While chemical modification of oligonucleotide internucleotide linkages with phosphorothioate, phosphorodithioate, and/or 5'-methylphosphonate linkages improves stability, excessive modifications can cause some toxicity or decreased activity. Therefore, when designing nucleic acid molecules, the amount of these internucleotide linkages should be minimized. The reduction in the concentration of these linkages should lower toxicity, resulting in increased efficacy and higher specificity of these molecules.

Short interfering nucleic acid (siNA) molecules having chemical modifications that maintain or enhance activity are provided. Such a nucleic acid is also generally more resistant to nucleases than an unmodified nucleic acid. Accordingly, the *in vitro* and/or *in vivo* activity should not be significantly lowered. In cases in which modulation is the goal, therapeutic nucleic acid molecules delivered exogenously should optimally be stable within cells until translation of the target RNA has been modulated long enough to reduce the levels of the undesirable protein. This period of time varies between hours to days depending upon the disease state. Improvements in the chemical synthesis of RNA and DNA (Wincott *et al.*, 1995, *Nucleic Acids Res.* 23, 2677; Caruthers *et al.*, 1992, *Methods in Enzymology* 211,3-19 (incorporated by reference herein)) have expanded the ability to modify nucleic acid molecules by introducing nucleotide modifications to enhance their nuclease stability, as described above.

In one embodiment, nucleic acid molecules of the invention include one or more (e.g., about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more) G-clamp nucleotides. A G-clamp nucleotide is a modified cytosine analog wherein the modifications confer the ability to hydrogen bond both Watson-Crick and Hoogsteen faces of a complementary guanine within a duplex, see for example Lin and Matteucci, 1998, *J. Am. Chem. Soc.*, 120, 8531-8532. A single G-clamp analog substitution within an oligonucleotide can result in substantially enhanced helical thermal stability and mismatch discrimination when hybridized to complementary oligonucleotides. The inclusion of such nucleotides in nucleic acid molecules of the invention results in both enhanced affinity and specificity to nucleic acid targets, complementary sequences, or template strands. In another embodiment, nucleic acid molecules of the invention include one or more (e.g., about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more) LNA "locked nucleic acid" nucleotides such as a 2', 4'-C methylene bicyclo nucleotide (see for example Wengel *et al.*, International PCT Publication No. WO 00/66604 and WO 99/14226).

In another embodiment, the invention features conjugates and/or complexes of siNA molecules of the invention. Such conjugates and/or complexes can be used to facilitate delivery of siNA molecules into a biological system, such as a cell. The conjugates and complexes provided by the instant invention can impart therapeutic activity by transferring therapeutic compounds across cellular membranes, altering the pharmacokinetics, and/or modulating the localization of nucleic acid molecules of the invention. The present invention encompasses the design and synthesis of novel conjugates and complexes for the delivery of molecules, including, but not limited to, small molecules, lipids, phospholipids, nucleosides, nucleotides, nucleic acids, antibodies, toxins, negatively charged polymers and other polymers, for example proteins, peptides, hormones, carbohydrates, polyethylene glycols, or polyamines, across cellular membranes. In general, the transporters described are designed to be used either individually or as part of a multi-component system, with or without degradable linkers. These compounds are expected to improve delivery and/or localization of nucleic acid molecules of the invention into a number of cell types originating from different tissues, in the presence or absence of serum (see Sullenger and Cech, U.S. Pat. No. 5,854,038). Conjugates of the molecules described herein can be attached to biologically active molecules via linkers that are biodegradable, such as biodegradable nucleic acid linker molecules.

The term "biodegradable linker" as used herein, refers to a nucleic acid or non-nucleic acid linker molecule that is designed as a biodegradable linker to connect one molecule to another molecule, for example, a biologically active molecule to a siNA molecule of the invention or the sense and antisense strands of a siNA molecule of the invention. The biodegradable linker is designed such that its stability can be modulated for a particular purpose, such as delivery to a particular tissue or cell type. The stability of a nucleic acid-based biodegradable linker molecule can be modulated by using various chemistries, for example combinations of ribonucleotides, deoxyribonucleotides, and chemically-modified nucleotides, such as 2'-O-methyl, 2'-fluoro, 2'-amino, 2'-O-amino, 2'-C-allyl, 2'-O-allyl, and other 2'-modified or base modified nucleotides. The biodegradable nucleic acid linker molecule can be a dimer, trimer, tetramer or longer nucleic acid molecule, for example, an oligonucleotide of about 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 nucleotides in length, or can comprise a single nucleotide with a phosphorus-based linkage, for example, a phosphoramidate or phosphodiester linkage. The biodegradable nucleic acid linker molecule can also comprise nucleic acid backbone, nucleic acid sugar, or nucleic acid base modifications.

The term "biodegradable" as used herein, refers to degradation in a biological system, for example enzymatic degradation or chemical degradation.

The term "biologically active molecule" as used herein, refers to compounds or molecules that are capable of eliciting or modifying a biological response in a system. Non-limiting examples of biologically active siNA molecules either alone or in combination with other molecules contemplated by the instant invention include therapeutically active molecules such as antibodies, hormones, antivirals, peptides, proteins, chemotherapeutics, small molecules, vitamins, co-factors, nucleosides, nucleotides, oligonucleotides, enzymatic nucleic acids, antisense nucleic acids, triplex forming oligonucleotides, 2,5-A chimeras, siNA, dsRNA, allozymes, aptamers, decoys and analogs thereof. Biologically active molecules of the invention also include molecules capable of modulating the pharmacokinetics and/or pharmacodynamics of other biologically active molecules, for example, lipids and polymers such as polyamines, polyamides, polyethylene glycol and other polyethers.

The term "phospholipid" as used herein, refers to a hydrophobic molecule comprising at least one phosphorus group. For example, a phospholipid can comprise a phosphorus-containing group and saturated or unsaturated alkyl group, optionally substituted with OH, COOH, oxo, amine, or substituted or unsubstituted aryl groups.

5 Therapeutic nucleic acid molecules (*e.g.*, siNA molecules) delivered exogenously optimally are stable within cells until reverse transcription of the RNA has been modulated long enough to reduce the levels of the RNA transcript. The nucleic acid molecules are resistant to nucleases in order to function as effective intracellular therapeutic agents. Improvements in the chemical synthesis of nucleic acid molecules
10 described in the instant invention and in the art have expanded the ability to modify nucleic acid molecules by introducing nucleotide modifications to enhance their nuclease stability as described above.

In yet another embodiment, siNA molecules having chemical modifications that maintain or enhance enzymatic activity of proteins involved in RNAi are provided. Such
15 nucleic acids are also generally more resistant to nucleases than unmodified nucleic acids. Thus, *in vitro* and/or *in vivo* the activity should not be significantly lowered.

Use of the nucleic acid-based molecules of the invention will lead to better treatment of the disease progression by affording the possibility of combination therapies (*e.g.*, multiple siNA molecules targeted to different genes; nucleic acid molecules
20 coupled with known small molecule modulators; or intermittent treatment with combinations of molecules, including different motifs and/or other chemical or biological molecules). The treatment of subjects with siNA molecules can also include combinations of different types of nucleic acid molecules, such as enzymatic nucleic acid molecules (ribozymes), allozymes, antisense, 2,5-A oligoadenylate, decoys, and
25 aptamers.

In another aspect a siNA molecule of the invention comprises one or more 5' and/or a 3'- cap structure, for example on only the sense siNA strand, the antisense siNA strand, or both siNA strands.

By "cap structure" is meant chemical modifications, which have been incorporated
30 at either terminus of the oligonucleotide (see, for example, Adamic *et al.*, U.S. Pat. No.

5,998,203, incorporated by reference herein). These terminal modifications protect the nucleic acid molecule from exonuclease degradation, and may help in delivery and/or localization within a cell. The cap may be present at the 5'-terminus (5'-cap) or at the 3'-terminal (3'-cap) or may be present on both termini. In non-limiting examples, the 5'-cap is selected from the group consisting of glyceryl, inverted deoxy abasic residue (moiety); 4',5'-methylene nucleotide; 1-(beta-D-erythrofuransyl) nucleotide, 4'-thio nucleotide; carbocyclic nucleotide; 1,5-anhydrohexitol nucleotide; L-nucleotides; alpha-nucleotides; modified base nucleotide; phosphorodithioate linkage; *threo*-pentofuransyl nucleotide; acyclic 3',4'-seco nucleotide; acyclic 3,4-dihydroxybutyl nucleotide; acyclic 3,5-dihydroxypentyl nucleotide, 3'-3'-inverted nucleotide moiety; 3'-3'-inverted abasic moiety; 3'-2'-inverted nucleotide moiety; 3'-2'-inverted abasic moiety; 1,4-butanediol phosphate; 3'-phosphoramidate; hexylphosphate; aminohexyl phosphate; 3'-phosphate; 3'-phosphorothioate; phosphorodithioate; or bridging or non-bridging methylphosphonate moiety.

15 In non-limiting examples, the 3'-cap is selected from the group consisting of glyceryl, inverted deoxy abasic residue (moiety), 4',5'-methylene nucleotide; 1-(beta-D-erythrofuransyl) nucleotide; 4'-thio nucleotide, carbocyclic nucleotide; 5'-amino-alkyl phosphate; 1,3-diamino-2-propyl phosphate; 3-aminopropyl phosphate; 6-aminohexyl phosphate; 1,2-aminododecyl phosphate; hydroxypropyl phosphate; 1,5-anhydrohexitol nucleotide; L-nucleotide; alpha-nucleotide; modified base nucleotide; phosphorodithioate; *threo*-pentofuransyl nucleotide; acyclic 3',4'-seco nucleotide; 3,4-dihydroxybutyl nucleotide; 3,5-dihydroxypentyl nucleotide, 5'-5'-inverted nucleotide moiety; 5'-5'-inverted abasic moiety; 5'-phosphoramidate; 5'-phosphorothioate; 1,4-butanediol phosphate; 5'-amino; bridging and/or non-bridging 5'-phosphoramidate, 25 phosphorothioate and/or phosphorodithioate, bridging or non bridging methylphosphonate and 5'-mercapto moieties (for more details see Beaucage and Iyer, 1993, *Tetrahedron* 49, 1925; incorporated by reference herein).

By the term "non-nucleotide" is meant any group or compound which can be incorporated into a nucleic acid chain in the place of one or more nucleotide units, including either sugar and/or phosphate substitutions, and allows the remaining bases to exhibit their enzymatic activity. The group or compound is abasic in that it does not

contain a commonly recognized nucleotide base, such as adenosine, guanine, cytosine, uracil or thymine and therefore lacks a base at the 1'-position.

An "alkyl" group refers to a saturated aliphatic hydrocarbon, including straight-chain, branched-chain, and cyclic alkyl groups. Preferably, the alkyl group has 1 to 12
5 carbons. More preferably, it is a lower alkyl of from 1 to 7 carbons, more preferably 1 to 4 carbons. The alkyl group can be substituted or unsubstituted. When substituted the substituted group(s) is preferably, hydroxyl, cyano, alkoxy, =O, =S, NO₂ or N(CH₃)₂, amino, or SH. The term also includes alkenyl groups that are unsaturated hydrocarbon groups containing at least one carbon-carbon double bond, including straight-chain,
10 branched-chain, and cyclic groups. Preferably, the alkenyl group has 1 to 12 carbons. More preferably, it is a lower alkenyl of from 1 to 7 carbons, more preferably 1 to 4 carbons. The alkenyl group may be substituted or unsubstituted. When substituted the substituted group(s) is preferably, hydroxyl, cyano, alkoxy, =O, =S, NO₂, halogen, N(CH₃)₂, amino, or SH. The term "alkyl" also includes alkynyl groups that have an
15 unsaturated hydrocarbon group containing at least one carbon-carbon triple bond, including straight-chain, branched-chain, and cyclic groups. Preferably, the alkynyl group has 1 to 12 carbons. More preferably, it is a lower alkynyl of from 1 to 7 carbons, more preferably 1 to 4 carbons. The alkynyl group may be substituted or unsubstituted. When substituted the substituted group(s) is preferably, hydroxyl, cyano, alkoxy, =O,
20 =S, NO₂ or N(CH₃)₂, amino or SH.

Such alkyl groups can also include aryl, alkylaryl, carbocyclic aryl, heterocyclic aryl, amide and ester groups. An "aryl" group refers to an aromatic group that has at least one ring having a conjugated pi electron system and includes carbocyclic aryl, heterocyclic aryl and biaryl groups, all of which may be optionally substituted. The
25 preferred substituent(s) of aryl groups are halogen, trihalomethyl, hydroxyl, SH, OH, cyano, alkoxy, alkyl, alkenyl, alkynyl, and amino groups. An "alkylaryl" group refers to an alkyl group (as described above) covalently joined to an aryl group (as described above). Carbocyclic aryl groups are groups wherein the ring atoms on the aromatic ring are all carbon atoms. The carbon atoms are optionally substituted. Heterocyclic aryl
30 groups are groups having from 1 to 3 heteroatoms as ring atoms in the aromatic ring and the remainder of the ring atoms are carbon atoms. Suitable heteroatoms include oxygen, sulfur, and nitrogen, and include furanyl, thienyl, pyridyl, pyrrolyl, N-lower alkyl

pyrrolo, pyrimidyl, pyrazinyl, imidazolyl and the like, all optionally substituted. An "amide" refers to an -C(O)-NH-R, where R is either alkyl, aryl, alkylaryl or hydrogen. An "ester" refers to an -C(O)-OR', where R is either alkyl, aryl, alkylaryl or hydrogen.

By "nucleotide" as used herein is as recognized in the art to include natural bases
5 (standard), and modified bases well known in the art. Such bases are generally located at the 1' position of a nucleotide sugar moiety. Nucleotides generally comprise a base, sugar and a phosphate group. The nucleotides can be unmodified or modified at the sugar, phosphate and/or base moiety, (also referred to interchangeably as nucleotide
10 analogs, modified nucleotides, non-natural nucleotides, non-standard nucleotides and other; see, for example, Usman and McSwiggen, *supra*; Eckstein *et al.*, International PCT Publication No. WO 92/07065; Usman *et al.*, International PCT Publication No. WO 93/15187; Uhlman & Peyman, *supra*, all are hereby incorporated by reference herein). There are several examples of modified nucleic acid bases known in the art as
15 summarized by Limbach *et al.*, 1994, *Nucleic Acids Res.* 22, 2183. Some of the non-limiting examples of base modifications that can be introduced into nucleic acid molecules include, inosine, purine, pyridin-4-one, pyridin-2-one, phenyl, pseudouracil, 2, 4, 6-trimethoxy benzene, 3-methyl uracil, dihydrouridine, naphthyl, aminophenyl, 5-alkylcytidines (*e.g.*, 5-methylcytidine), 5-alkyluridines (*e.g.*, ribothymidine), 5-halouridine (*e.g.*, 5-bromouridine) or 6-azapyrimidines or 6-alkylpyrimidines (*e.g.* 6-
20 methyluridine), propyne, and others (Burgin *et al.*, 1996, *Biochemistry*, 35, 14090; Uhlman & Peyman, *supra*). By "modified bases" in this aspect is meant nucleotide bases other than adenine, guanine, cytosine and uracil at 1' position or their equivalents.

In one embodiment, the invention features modified siNA molecules, with phosphate backbone modifications comprising one or more phosphorothioate,
25 phosphorodithioate, methylphosphonate, phosphotriester, morpholino, amidate carbamate, carboxymethyl, acetamidate, polyamide, sulfonate, sulfonamide, sulfamate, formacetal, thioformacetal, and/or alkylsilyl, substitutions. For a review of oligonucleotide backbone modifications, see Hunziker and Leumann, 1995, *Nucleic Acid Analogues: Synthesis and Properties*, in *Modern Synthetic Methods*, VCH, 331-417, and
30 Mesmaeker *et al.*, 1994, *Novel Backbone Replacements for Oligonucleotides*, in *Carbohydrate Modifications in Antisense Research*, ACS, 24-39.

By "abasic" is meant sugar moieties lacking a base or having other chemical groups in place of a base at the 1' position, see for example Adamic *et al.*, U.S. Pat. No. 5,998,203.

By "unmodified nucleoside" is meant one of the bases adenine, cytosine, guanine, thymine, or uracil joined to the 1' carbon of β -D-ribo-furanose.

By "modified nucleoside" is meant any nucleotide base which contains a modification in the chemical structure of an unmodified nucleotide base, sugar and/or phosphate. Non-limiting examples of modified nucleotides are shown by Formulae I-VII and/or other modifications described herein.

In connection with 2'-modified nucleotides as described for the present invention, by "amino" is meant 2'-NH₂ or 2'-O- NH₂, which can be modified or unmodified. Such modified groups are described, for example, in Eckstein *et al.*, U.S. Pat. No. 5,672,695 and Matulic-Adamic *et al.*, U.S. Pat. No. 6,248,878, which are both incorporated by reference in their entireties.

Various modifications to nucleic acid siNA structure can be made to enhance the utility of these molecules. Such modifications will enhance shelf-life, half-life *in vitro*, stability, and ease of introduction of such oligonucleotides to the target site, *e.g.*, to enhance penetration of cellular membranes, and confer the ability to recognize and bind to targeted cells.

Administration of Nucleic Acid Molecules

A siRNA molecule of the invention can be adapted for use to treat for example HCV infection, liver failure, hepatocellular carcinoma, cirrhosis and other indications that can respond to the level of HCV in a cell or tissue, alone or in combination with other therapies. For example, a siNA molecule can comprise a delivery vehicle, including liposomes, for administration to a subject, carriers and diluents and their salts, and/or can be present in pharmaceutically acceptable formulations. Methods for the delivery of nucleic acid molecules are described in Akhtar *et al.*, 1992, *Trends Cell Bio.*, 2, 139; *Delivery Strategies for Antisense Oligonucleotide Therapeutics*, ed. Akhtar, 1995, Maurer *et al.*, 1999, *Mol. Membr. Biol.*, 16, 129-140; Hofland and Huang, 1999, *Handb. Exp. Pharmacol.*, 137, 165-192; and Lee *et al.*, 2000, *ACS Symp. Ser.*, 752, 184-

192, all of which are incorporated herein by reference. Beigelman *et al.*, U.S. Pat. No. 6,395,713 and Sullivan *et al.*, PCT WO 94/02595 further describe the general methods for delivery of nucleic acid molecules. These protocols can be utilized for the delivery of virtually any nucleic acid molecule. Nucleic acid molecules can be administered to cells
5 by a variety of methods known to those of skill in the art, including, but not restricted to, encapsulation in liposomes, by iontophoresis, or by incorporation into other vehicles, such as hydrogels, cyclodextrins (see for example Gonzalez *et al.*, 1999, *Bioconjugate Chem.*, 10, 1068-1074), biodegradable nanocapsules, and bioadhesive microspheres, or by proteinaceous vectors (O'Hare and Normand, International PCT Publication No. WO
10 00/53722). Alternatively, the nucleic acid/vehicle combination is locally delivered by direct injection or by use of an infusion pump. Direct injection of the nucleic acid molecules of the invention, whether subcutaneous, intramuscular, or intradermal, can take place using standard needle and syringe methodologies, or by needle-free technologies such as those described in Conry *et al.*, 1999, *Clin. Cancer Res.*, 5, 2330-
15 2337 and Barry *et al.*, International PCT Publication No. WO 99/31262. The molecules of the instant invention can be used as pharmaceutical agents. Pharmaceutical agents prevent, modulate the occurrence, or treat (alleviate a symptom to some extent, preferably all of the symptoms) of a disease state in a subject.

Thus, the invention features a pharmaceutical composition comprising one or more
20 nucleic acid(s) of the invention in an acceptable carrier, such as a stabilizer, buffer, and the like. The polynucleotides of the invention can be administered (*e.g.*, RNA, DNA or protein) and introduced into a subject by any standard means, with or without stabilizers, buffers, and the like, to form a pharmaceutical composition. When it is desired to use a liposome delivery mechanism, standard protocols for formation of liposomes can be
25 followed. The compositions of the present invention can also be formulated and used as tablets, capsules or elixirs for oral administration, suppositories for rectal administration, sterile solutions, suspensions for injectable administration, and the other compositions known in the art.

The present invention also includes pharmaceutically acceptable formulations of
30 the compounds described. These formulations include salts of the above compounds, *e.g.*, acid addition salts, for example, salts of hydrochloric, hydrobromic, acetic acid, and benzene sulfonic acid.

A pharmacological composition or formulation refers to a composition or formulation in a form suitable for administration, *e.g.*, systemic administration, into a cell or subject, including for example a human. Suitable forms, in part, depend upon the use or the route of entry, for example oral, transdermal, or by injection. Such forms
5 should not prevent the composition or formulation from reaching a target cell (*i.e.*, a cell to which the negatively charged nucleic acid is desirable for delivery). For example, pharmacological compositions injected into the blood stream should be soluble. Other factors are known in the art, and include considerations such as toxicity and forms that prevent the composition or formulation from exerting its effect.

10 By "systemic administration" is meant *in vivo* systemic absorption or accumulation of drugs in the blood stream followed by distribution throughout the entire body. Administration routes that lead to systemic absorption include, without limitation: intravenous, subcutaneous, intraperitoneal, inhalation, oral, intrapulmonary and intramuscular. Each of these administration routes exposes the siNA molecules of the
15 invention to an accessible diseased tissue. The rate of entry of a drug into the circulation has been shown to be a function of molecular weight or size. The use of a liposome or other drug carrier comprising the compounds of the instant invention can potentially localize the drug, for example, in certain tissue types, such as the tissues of the reticular endothelial system (RES). A liposome formulation that can facilitate the
20 association of drug with the surface of cells, such as, lymphocytes and macrophages is also useful. This approach can provide enhanced delivery of the drug to target cells by taking advantage of the specificity of macrophage and lymphocyte immune recognition of abnormal cells, such as cells producing excess HCV.

By "pharmaceutically acceptable formulation" is meant, a composition or
25 formulation that allows for the effective distribution of the nucleic acid molecules of the instant invention in the physical location most suitable for their desired activity. Non-limiting examples of agents suitable for formulation with the nucleic acid molecules of the instant invention include: P-glycoprotein inhibitors (such as Pluronic P85), which can enhance entry of drugs into the CNS (Joliet-Riant and Tillement, 1999, *Fundam. Clin.*
30 *Pharmacol.*, 13, 16-26); biodegradable polymers, such as poly (DL-lactide-coglycolide) microspheres for sustained release delivery after intracerebral implantation (Emerich, DF *et al*, 1999, *Cell Transplant*, 8, 47-58) (Alkermes, Inc. Cambridge, MA); and loaded

nanoparticles, such as those made of polybutylcyanoacrylate, which can deliver drugs across the blood brain barrier and can alter neuronal uptake mechanisms (*Prog Neuropsychopharmacol Biol Psychiatry*, 23, 941-949, 1999). Other non-limiting examples of delivery strategies for the nucleic acid molecules of the instant invention
5 include material described in Boado *et al.*, 1998, *J. Pharm. Sci.*, 87, 1308-1315; Tyler *et al.*, 1999, *FEBS Lett.*, 421, 280-284; Pardridge *et al.*, 1995, *PNAS USA.*, 92, 5592-5596; Boado, 1995, *Adv. Drug Delivery Rev.*, 15, 73-107; Aldrian-Herrada *et al.*, 1998, *Nucleic Acids Res.*, 26, 4910-4916; and Tyler *et al.*, 1999, *PNAS USA.*, 96, 7053-7058.

The invention also features the use of the composition comprising surface-
10 modified liposomes containing poly (ethylene glycol) lipids (PEG-modified, or long-circulating liposomes or stealth liposomes). These formulations offer a method for increasing the accumulation of drugs in target tissues. This class of drug carriers resists opsonization and elimination by the mononuclear phagocytic system (MPS or RES), thereby enabling longer blood circulation times and enhanced tissue exposure for the
15 encapsulated drug (Lasic *et al.* *Chem. Rev.* 1995, 95, 2601-2627; Ishiwata *et al.*, *Chem. Pharm. Bull.* 1995, 43, 1005-1011). Such liposomes have been shown to accumulate selectively in tumors, presumably by extravasation and capture in the neovascularized target tissues (Lasic *et al.*, *Science* 1995, 267, 1275-1276; Oku *et al.*, 1995, *Biochim. Biophys. Acta*, 1238, 86-90). The long-circulating liposomes enhance the
20 pharmacokinetics and pharmacodynamics of DNA and RNA, particularly compared to conventional cationic liposomes which are known to accumulate in tissues of the MPS (Liu *et al.*, *J. Biol. Chem.* 1995, 270, 24864-24870; Choi *et al.*, International PCT Publication No. WO 96/10391; Ansell *et al.*, International PCT Publication No. WO 96/10390; Holland *et al.*, International PCT Publication No. WO 96/10392). Long-
25 circulating liposomes are also likely to protect drugs from nuclease degradation to a greater extent compared to cationic liposomes, based on their ability to avoid accumulation in metabolically aggressive MPS tissues such as the liver and spleen.

The present invention also includes compositions prepared for storage or administration that include a pharmaceutically effective amount of the desired
30 compounds in a pharmaceutically acceptable carrier or diluent. Acceptable carriers or diluents for therapeutic use are well known in the pharmaceutical art, and are described, for example, in *Remington's Pharmaceutical Sciences*, Mack Publishing Co. (A.R.

Gennaro edit. 1985), hereby incorporated by reference herein. For example, preservatives, stabilizers, dyes and flavoring agents can be provided. These include sodium benzoate, sorbic acid and esters of *p*-hydroxybenzoic acid. In addition, antioxidants and suspending agents can be used.

5 A pharmaceutically effective dose is that dose required to prevent, inhibit the occurrence, or treat (alleviate a symptom to some extent, preferably all of the symptoms) of a disease state. The pharmaceutically effective dose depends on the type of disease, the composition used, the route of administration, the type of mammal being treated, the physical characteristics of the specific mammal under consideration, concurrent
10 medication, and other factors that those skilled in the medical arts will recognize. Generally, an amount between 0.1 mg/kg and 100 mg/kg body weight/day of active ingredients is administered dependent upon potency of the negatively charged polymer.

The nucleic acid molecules of the invention and formulations thereof can be administered orally, topically, parenterally, by inhalation or spray, or rectally in dosage
15 unit formulations containing conventional non-toxic pharmaceutically acceptable carriers, adjuvants and/or vehicles. The term parenteral as used herein includes percutaneous, subcutaneous, intravascular (*e.g.*, intravenous), intramuscular, or intrathecal injection or infusion techniques and the like. In addition, there is provided a pharmaceutical formulation comprising a nucleic acid molecule of the invention and a
20 pharmaceutically acceptable carrier. One or more nucleic acid molecules of the invention can be present in association with one or more non-toxic pharmaceutically acceptable carriers and/or diluents and/or adjuvants, and if desired other active ingredients. The pharmaceutical compositions containing nucleic acid molecules of the invention can be in a form suitable for oral use, for example, as tablets, troches,
25 lozenges, aqueous or oily suspensions, dispersible powders or granules, emulsion, hard or soft capsules, or syrups or elixirs.

Compositions intended for oral use can be prepared according to any method known to the art for the manufacture of pharmaceutical compositions and such compositions can contain one or more such sweetening agents, flavoring agents, coloring
30 agents or preservative agents in order to provide pharmaceutically elegant and palatable preparations. Tablets contain the active ingredient in admixture with non-toxic

pharmaceutically acceptable excipients that are suitable for the manufacture of tablets. These excipients can be, for example, inert diluents; such as calcium carbonate, sodium carbonate, lactose, calcium phosphate or sodium phosphate; granulating and disintegrating agents, for example, corn starch, or alginic acid; binding agents, for example starch, gelatin or acacia; and lubricating agents, for example magnesium stearate, stearic acid or talc. The tablets can be uncoated or they can be coated by known techniques. In some cases such coatings can be prepared by known techniques to delay disintegration and absorption in the gastrointestinal tract and thereby provide a sustained action over a longer period. For example, a time delay material such as glyceryl monostearate or glyceryl distearate can be employed.

Formulations for oral use can also be presented as hard gelatin capsules wherein the active ingredient is mixed with an inert solid diluent, for example, calcium carbonate, calcium phosphate or kaolin, or as soft gelatin capsules wherein the active ingredient is mixed with water or an oil medium, for example peanut oil, liquid paraffin or olive oil.

Aqueous suspensions contain the active materials in a mixture with excipients suitable for the manufacture of aqueous suspensions. Such excipients are suspending agents, for example sodium carboxymethylcellulose, methylcellulose, hydropropylmethylcellulose, sodium alginate, polyvinylpyrrolidone, gum tragacanth and gum acacia; dispersing or wetting agents can be a naturally-occurring phosphatide, for example, lecithin, or condensation products of an alkylene oxide with fatty acids, for example polyoxyethylene stearate, or condensation products of ethylene oxide with long chain aliphatic alcohols, for example heptadecaethyleneoxycetanol, or condensation products of ethylene oxide with partial esters derived from fatty acids and a hexitol such as polyoxyethylene sorbitol monooleate, or condensation products of ethylene oxide with partial esters derived from fatty acids and hexitol anhydrides, for example polyethylene sorbitan monooleate. The aqueous suspensions can also contain one or more preservatives, for example ethyl, or n-propyl p-hydroxybenzoate, one or more coloring agents, one or more flavoring agents, and one or more sweetening agents, such as sucrose or saccharin.

Oily suspensions can be formulated by suspending the active ingredients in a vegetable oil, for example arachis oil, olive oil, sesame oil or coconut oil, or in a mineral

oil such as liquid paraffin. The oily suspensions can contain a thickening agent, for example beeswax, hard paraffin or cetyl alcohol. Sweetening agents and flavoring agents can be added to provide palatable oral preparations. These compositions can be preserved by the addition of an anti-oxidant such as ascorbic acid

5 Dispersible powders and granules suitable for preparation of an aqueous suspension by the addition of water provide the active ingredient in admixture with a dispersing or wetting agent, suspending agent and one or more preservatives. Suitable dispersing or wetting agents or suspending agents are exemplified by those already mentioned above. Additional excipients, for example sweetening, flavoring and coloring
10 agents, can also be present.

Pharmaceutical compositions of the invention can also be in the form of oil-in-water emulsions. The oily phase can be a vegetable oil or a mineral oil or mixtures of these. Suitable emulsifying agents can be naturally-occurring gums, for example gum acacia or gum tragacanth, naturally-occurring phosphatides, for example soy bean,
15 lecithin, and esters or partial esters derived from fatty acids and hexitol, anhydrides, for example sorbitan monooleate, and condensation products of the said partial esters with ethylene oxide, for example polyoxyethylene sorbitan monooleate. The emulsions can also contain sweetening and flavoring agents.

Syrups and elixirs can be formulated with sweetening agents, for example glycerol,
20 propylene glycol, sorbitol, glucose or sucrose. Such formulations can also contain a demulcent, a preservative and flavoring and coloring agents. The pharmaceutical compositions can be in the form of a sterile injectable aqueous or oleaginous suspension. This suspension can be formulated according to the known art using those suitable dispersing or wetting agents and suspending agents that have been mentioned above.
25 The sterile injectable preparation can also be a sterile injectable solution or suspension in a non-toxic parentally acceptable diluent or solvent, for example as a solution in 1,3-butanediol. Among the acceptable vehicles and solvents that can be employed are water, Ringer's solution and isotonic sodium chloride solution. In addition, sterile, fixed oils are conventionally employed as a solvent or suspending medium. For this purpose, any
30 bland fixed oil can be employed including synthetic mono-or diglycerides. In addition, fatty acids such as oleic acid find use in the preparation of injectables.

The nucleic acid molecules of the invention can also be administered in the form of suppositories, *e.g.*, for rectal administration of the drug. These compositions can be prepared by mixing the drug with a suitable non-irritating excipient that is solid at ordinary temperatures but liquid at the rectal temperature and will therefore melt in the rectum to release the drug. Such materials include cocoa butter and polyethylene glycols.

Nucleic acid molecules of the invention can be administered parenterally in a sterile medium. The drug, depending on the vehicle and concentration used, can either be suspended or dissolved in the vehicle. Advantageously, adjuvants such as local anesthetics, preservatives and buffering agents can be dissolved in the vehicle.

Dosage levels of the order of from about 0.1 mg to about 140 mg per kilogram of body weight per day are useful in the treatment of the above-indicated conditions (about 0.5 mg to about 7 g per subject per day). The amount of active ingredient that can be combined with the carrier materials to produce a single dosage form varies depending upon the host treated and the particular mode of administration. Dosage unit forms generally contain between from about 1 mg to about 500 mg of an active ingredient.

It is understood that the specific dose level for any particular subject depends upon a variety of factors including the activity of the specific compound employed, the age, body weight, general health, sex, diet, time of administration, route of administration, and rate of excretion, drug combination and the severity of the particular disease undergoing therapy.

For administration to non-human animals, the composition can also be added to the animal feed or drinking water. It can be convenient to formulate the animal feed and drinking water compositions so that the animal takes in a therapeutically appropriate quantity of the composition along with its diet. It can also be convenient to present the composition as a premix for addition to the feed or drinking water.

The nucleic acid molecules of the present invention can also be administered to a subject in combination with other therapeutic compounds to increase the overall therapeutic effect. The use of multiple compounds to treat an indication can increase the beneficial effects while reducing the presence of side effects.

In one embodiment, the invention comprises compositions suitable for administering nucleic acid molecules of the invention to specific cell types. For example, the asialoglycoprotein receptor (ASGPr) (Wu and Wu, 1987, *J. Biol. Chem.* 262, 4429-4432) is unique to hepatocytes and binds branched galactose-terminal glycoproteins, such as asialoorosomucoid (ASOR). In another example, the folate receptor is overexpressed in many cancer cells. Binding of such glycoproteins, synthetic glycoconjugates, or folates to the receptor takes place with an affinity that strongly depends on the degree of branching of the oligosaccharide chain, for example, triantennary structures are bound with greater affinity than biantennary or monoantennary chains (Baenziger and Fiete, 1980, *Cell*, 22, 611-620; Connolly *et al.*, 1982, *J. Biol. Chem.*, 257, 939-945). Lee and Lee, 1987, *Glycoconjugate J.*, 4, 317-328, obtained this high specificity through the use of N-acetyl-D-galactosamine as the carbohydrate moiety, which has higher affinity for the receptor, compared to galactose. This "clustering effect" has also been described for the binding and uptake of mannosyl-terminating glycoproteins or glycoconjugates (Ponpipom *et al.*, 1981, *J. Med. Chem.*, 24, 1388-1395). The use of galactose, galactosamine, or folate based conjugates to transport exogenous compounds across cell membranes can provide a targeted delivery approach to, for example, the treatment of liver disease, cancers of the liver, or other cancers. The use of bioconjugates can also provide a reduction in the required dose of therapeutic compounds required for treatment. Furthermore, therapeutic bioavailability, pharmacodynamics, and pharmacokinetic parameters can be modulated through the use of nucleic acid bioconjugates of the invention. Non-limiting examples of such bioconjugates are described in Vargeese *et al.*, USSN 10/201,394, filed August 13, 2001; and Matulic-Adamic *et al.*, USSN 60/362,016, filed March 6, 2002.

Alternatively, certain siNA molecules of the instant invention can be expressed within cells from eukaryotic promoters (e.g., Izant and Weintraub, 1985, *Science*, 229, 345; McGarry and Lindquist, 1986, *Proc. Natl. Acad. Sci.*, USA 83, 399; Scanlon *et al.*, 1991, *Proc. Natl. Acad. Sci. USA*, 88, 10591-5; Kashani-Sabet *et al.*, 1992, *Antisense Res. Dev.*, 2, 3-15; Dropulic *et al.*, 1992, *J. Virol.*, 66, 1432-41; Weerasinghe *et al.*, 1991, *J. Virol.*, 65, 5531-4; Ojwang *et al.*, 1992, *Proc. Natl. Acad. Sci. USA*, 89, 10802-6; Chen *et al.*, 1992, *Nucleic Acids Res.*, 20, 4581-9; Sarver *et al.*, 1990 *Science*, 247, 1222-1225; Thompson *et al.*, 1995, *Nucleic Acids Res.*, 23, 2259; Good *et al.*, 1997, *Gene Therapy*, 4, 45. Those skilled in the art realize that any nucleic acid can be

expressed in eukaryotic cells from the appropriate DNA/RNA vector. The activity of such nucleic acids can be augmented by their release from the primary transcript by a enzymatic nucleic acid (Draper *et al.*, PCT WO 93/23569, and Sullivan *et al.*, PCT WO 94/02595; Ohkawa *et al.*, 1992, *Nucleic Acids Symp. Ser.*, 27, 15-6; Taira *et al.*, 1991, 5 *Nucleic Acids Res.*, 19, 5125-30; Ventura *et al.*, 1993, *Nucleic Acids Res.*, 21, 3249-55; Chowrira *et al.*, 1994, *J. Biol. Chem.*, 269, 25856.

In another aspect of the invention, RNA molecules of the present invention can be expressed from transcription units (see for example Couture *et al.*, 1996, *TIG.*, 12, 510) inserted into DNA or RNA vectors. The recombinant vectors can be DNA plasmids or 10 viral vectors. siNA expressing viral vectors can be constructed based on, but not limited to, adeno-associated virus, retrovirus, adenovirus, or alphavirus. In another embodiment, pol III based constructs are used to express nucleic acid molecules of the invention (see for example Thompson, U.S. Pats. Nos. 5,902,880 and 6,146,886). The recombinant vectors capable of expressing the siNA molecules can be delivered as described above, 15 and persist in target cells. Alternatively, viral vectors can be used that provide for transient expression of nucleic acid molecules. Such vectors can be repeatedly administered as necessary. Once expressed, the siNA molecule interacts with the target mRNA and generates an RNAi response. Delivery of siNA molecule expressing vectors can be systemic, such as by intravenous or intra-muscular administration, by 20 administration to target cells ex-planted from a subject followed by reintroduction into the subject, or by any other means that would allow for introduction into the desired target cell (for a review see Couture *et al.*, 1996, *TIG.*, 12, 510).

In one aspect the invention features an expression vector comprising a nucleic acid sequence encoding at least one siNA molecule of the instant invention. The expression 25 vector can encode one or both strands of a siNA duplex, or a single self-complementary strand that self hybridizes into a siNA duplex. The nucleic acid sequences encoding the siNA molecules of the instant invention can be operably linked in a manner that allows expression of the siNA molecule (see for example Paul *et al.*, 2002, *Nature Biotechnology*, 19, 505; Miyagishi and Taira, 2002, *Nature Biotechnology*, 19, 497; Lee 30 *et al.*, 2002, *Nature Biotechnology*, 19, 500; and Novina *et al.*, 2002, *Nature Medicine*, advance online publication doi:10.1038/nm725).

In another aspect, the invention features an expression vector comprising: a) a transcription initiation region (*e.g.*, eukaryotic pol I, II or III initiation region); b) a transcription termination region (*e.g.*, eukaryotic pol I, II or III termination region); and c) a nucleic acid sequence encoding at least one of the siNA molecules of the instant invention; wherein said sequence is operably linked to said initiation region and said termination region, in a manner that allows expression and/or delivery of the siNA molecule. The vector can optionally include an open reading frame (ORF) for a protein operably linked on the 5' side or the 3'-side of the sequence encoding the siNA of the invention; and/or an intron (intervening sequences).

Transcription of the siNA molecule sequences can be driven from a promoter for eukaryotic RNA polymerase I (pol I), RNA polymerase II (pol II), or RNA polymerase III (pol III). Transcripts from pol II or pol III promoters are expressed at high levels in all cells; the levels of a given pol II promoter in a given cell type depends on the nature of the gene regulatory sequences (enhancers, silencers, etc.) present nearby. Prokaryotic RNA polymerase promoters are also used, providing that the prokaryotic RNA polymerase enzyme is expressed in the appropriate cells (Elroy-Stein and Moss, 1990, *Proc. Natl. Acad. Sci. U S A*, 87, 6743-7; Gao and Huang 1993, *Nucleic Acids Res.*, 21, 2867-72; Lieber *et al.*, 1993, *Methods Enzymol.*, 217, 47-66; Zhou *et al.*, 1990, *Mol. Cell. Biol.*, 10, 4529-37). Several investigators have demonstrated that nucleic acid molecules expressed from such promoters can function in mammalian cells (*e.g.* Kashani-Sabet *et al.*, 1992, *Antisense Res. Dev.*, 2, 3-15; Ojwang *et al.*, 1992, *Proc. Natl. Acad. Sci. U S A*, 89, 10802-6; Chen *et al.*, 1992, *Nucleic Acids Res.*, 20, 4581-9; Yu *et al.*, 1993, *Proc. Natl. Acad. Sci. U S A*, 90, 6340-4; L'Huillier *et al.*, 1992, *EMBO J.*, 11, 4411-8; Lisiewicz *et al.*, 1993, *Proc. Natl. Acad. Sci. U. S. A*, 90, 8000-4; Thompson *et al.*, 1995, *Nucleic Acids Res.*, 23, 2259; Sullenger & Cech, 1993, *Science*, 262, 1566). More specifically, transcription units such as the ones derived from genes encoding U6 small nuclear (snRNA), transfer RNA (tRNA) and adenovirus VA RNA are useful in generating high concentrations of desired RNA molecules such as siNA in cells (Thompson *et al.*, *supra*; Couture and Stinchcomb, 1996, *supra*; Noonberg *et al.*, 1994, *Nucleic Acid Res.*, 22, 2830; Noonberg *et al.*, U.S. Pat. No. 5,624,803; Good *et al.*, 1997, *Gene Ther.*, 4, 45; Beigelman *et al.*, International PCT Publication No. WO 96/18736. The above siNA transcription units can be incorporated into a variety of vectors for introduction into mammalian cells, including but not restricted to, plasmid DNA vectors,

viral DNA vectors (such as adenovirus or adeno-associated virus vectors), or viral RNA vectors (such as retroviral or alphavirus vectors) (for a review see Couture and Stinchcomb, 1996, *supra*).

5 In another aspect the invention features an expression vector comprising a nucleic acid sequence encoding at least one of the siNA molecules of the invention in a manner that allows expression of that siNA molecule. The expression vector comprises in one embodiment; a) a transcription initiation region; b) a transcription termination region; and c) a nucleic acid sequence encoding at least one strand of the siNA molecule, wherein the sequence is operably linked to the initiation region and the termination
10 region in a manner that allows expression and/or delivery of the siNA molecule.

In another embodiment the expression vector comprises: a) a transcription initiation region; b) a transcription termination region; c) an open reading frame; and d) a nucleic acid sequence encoding at least one strand of a siNA molecule, wherein the sequence is operably linked to the 3'-end of the open reading frame and wherein the
15 sequence is operably linked to the initiation region, the open reading frame and the termination region in a manner that allows expression and/or delivery of the siNA molecule. In yet another embodiment, the expression vector comprises: a) a transcription initiation region; b) a transcription termination region; c) an intron; and d) a nucleic acid sequence encoding at least one siNA molecule, wherein the sequence is
20 operably linked to the initiation region, the intron and the termination region in a manner which allows expression and/or delivery of the nucleic acid molecule.

In another embodiment, the expression vector comprises: a) a transcription initiation region; b) a transcription termination region; c) an intron; d) an open reading frame; and e) a nucleic acid sequence encoding at least one strand of a siNA molecule,
25 wherein the sequence is operably linked to the 3'-end of the open reading frame and wherein the sequence is operably linked to the initiation region, the intron, the open reading frame and the termination region in a manner which allows expression and/or delivery of the siNA molecule.

HCV biology and biochemistry

In 1989, the Hepatitis C Virus (HCV) was determined to be an RNA virus and was identified as the causative agent of most non-A non-B viral Hepatitis (Choo *et al.*, 1989, *Science*, 244, 359-362). Unlike retroviruses such as HIV, HCV does not go through a DNA replication phase and no integrated forms of the viral genome into the host chromosome have been detected (Houghton *et al.*, 1991, *Hepatology*, 14, 381-388). Rather, replication of the coding (plus) strand is mediated by the production of a replicative (minus) strand leading to the generation of several copies of plus strand HCV RNA. The genome consists of a single, large, open-reading frame that is translated into a polyprotein (Kato *et al.*, 1991, *FEBS Letters*, 280: 325-328). This polyprotein subsequently undergoes post-translational cleavage, producing several viral proteins (Leinbach *et al.*, 1994, *Virology*, 204:163-169).

Examination of the 9.5-kilobase genome of HCV has demonstrated that the viral nucleic acid can mutate at a high rate (Smith *et al.*, 1997 *Mol. Evol.* 45, 238-246). This rate of mutation has led to the evolution of several distinct genotypes of HCV that share approximately 70% sequence identity (Simmonds *et al.*, 1994, *J. Gen. Virol.* 75, 1053-1061). It is important to note that these sequences are evolutionarily quite distant. For example, the genetic identity between humans and primates such as the chimpanzee is approximately 98%. In addition, it has been demonstrated that an HCV infection in an individual patient is composed of several distinct and evolving quasispecies that have 98% identity at the RNA level. Thus, the HCV genome is hypervariable and continuously changing. Although the HCV genome is hypervariable, there are 3 regions of the genome that are highly conserved. These conserved sequences occur in the 5' and 3' non-coding regions as well as the 5'-end of the core protein coding region and are thought to be vital for HCV RNA replication as well as translation of the HCV polyprotein. Thus, therapeutic agents that target these conserved HCV genomic regions may have a significant impact over a wide range of HCV genotypes. Moreover, it is unlikely that drug resistance will occur with enzymatic nucleic acids specific to conserved regions of the HCV genome. In contrast, therapeutic modalities that target inhibition of enzymes such as the viral proteases or helicase are likely to result in the selection for drug resistant strains since the RNA for these viral encoded enzymes is located in the hypervariable portion of the HCV genome.

After initial exposure to HCV, a patient experiences a transient rise in liver enzymes, which indicates that inflammatory processes are occurring (Alter *et al.*, IN: Seeff LB, Lewis JH, eds. *Current Perspectives in Hepatology*. New York: Plenum Medical Book Co; 1989:83-89). This elevation in liver enzymes occurs at least 4 weeks
5 after the initial exposure and may last for up to two months (Farci *et al.*, 1991, *New England Journal of Medicine*. 325, 98-104). Prior to the rise in liver enzymes, it is possible to detect HCV RNA in the patient's serum using RT-PCR analysis (Takahashi *et al.*, 1993, *American Journal of Gastroenterology*. 88, 240-243). This stage of the disease is called the acute stage and usually goes undetected since 75% of patients with acute
10 viral hepatitis from HCV infection are asymptomatic. The remaining 25% of these patients develop jaundice or other symptoms of hepatitis.

Although acute HCV infection is a benign disease, as many as 80% of acute HCV patients progress to chronic liver disease as evidenced by persistent elevation of serum alanine aminotransferase (ALT) levels and by continual presence of circulating HCV
15 RNA (Sherlock, 1992, *Lancet*, 339, 802). The natural progression of chronic HCV infection over a 10 to 20 year period leads to cirrhosis in 20 to 50% of patients (Davis *et al.*, 1993, *Infectious Agents and Disease*, 2, 150, 154) and progression of HCV infection to hepatocellular carcinoma has been well documented (Liang *et al.*, 1993, *Hepatology*. 18, 1326-1333; Tong *et al.*, 1994, *Western Journal of Medicine*, 160, 133-138). There
20 have been no studies that have determined sub-populations that are most likely to progress to cirrhosis and/or hepatocellular carcinoma, thus all patients have equal risk of progression.

It is important to note that the survival for patients diagnosed with hepatocellular carcinoma is only 0.9 to 12.8 months from initial diagnosis (Takahashi *et al.*, 1993,
25 *American Journal of Gastroenterology*. 88, 240-243). Treatment of hepatocellular carcinoma with chemotherapeutic agents has not proven effective and only 10% of patients will benefit from surgery due to extensive tumor invasion of the liver (Trinchet *et al.*, 1994, *Presse Medicin*. 23, 831-833). Given the aggressive nature of primary hepatocellular carcinoma, the only viable treatment alternative to surgery is liver
30 transplantation (Pichlmayr *et al.*, 1994, *Hepatology*. 20, 33S-40S).

Upon progression to cirrhosis, patients with chronic HCV infection present with clinical features, which are common to clinical cirrhosis regardless of the initial cause (D'Amico *et al.*, 1986, *Digestive Diseases and Sciences*. 31, 468-475). These clinical features may include: bleeding esophageal varices, ascites, jaundice, and encephalopathy (Zakim D, Boyer TD. *Hepatology a textbook of liver disease*. Second Edition Volume 1. 1990 W.B. Saunders Company. Philadelphia). In the early stages of cirrhosis, patients are classified as compensated, the stage at which the patient's liver is still able to detoxify metabolites in the blood-stream although liver tissue damage has occurred. In addition, most patients with compensated liver disease are asymptomatic and the minority with symptoms report only minor symptoms, such as dyspepsia and weakness. In the later stages of cirrhosis, patients are classified as decompensated, the stage at which the ability of the liver to detoxify metabolites in the bloodstream is diminished. It is at the decompensated stage that the clinical features described above present.

In 1986, D'Amico *et al.* described the clinical manifestations and survival rates in 1155 patients with both alcoholic and viral associated cirrhosis (D'Amico *supra*). Of the 1155 patients, 435 (37%) had compensated disease although 70% were asymptomatic at the beginning of the study. The remaining 720 patients (63%) had decompensated liver disease with 78% presenting with a history of ascites, 31% with jaundice, 17% had bleeding and 16% had encephalopathy. Hepatocellular carcinoma was observed in six (.5%) patients with compensated disease and in 30 (2.6%) patients with decompensated disease.

Over the course of six years, the patients with compensated cirrhosis developed clinical features of decompensated disease at a rate of 10% per year. In most cases, ascites was the first presentation of decompensation. In addition, hepatocellular carcinoma developed in 59 patients who initially presented with compensated disease by the end of the six-year study.

With respect to survival, the D'Amico study indicated that the five-year survival rate for all patients in the study was only 40%. The six-year survival rate for the patients who initially had compensated cirrhosis was 54% while the six-year survival rate for patients who initially presented with decompensated disease was only 21%. There were no significant differences in the survival rates between the patients who had alcoholic

cirrhosis and the patients with viral related cirrhosis. The major causes of death for the patients in the D'Amico study were liver failure in 49%; hepatocellular carcinoma in 22%; and bleeding in 13% (D'Amico *supra*).

Chronic Hepatitis C is a slowly progressing inflammatory disease of the liver, mediated by a virus (HCV) that can lead to cirrhosis, liver failure and/or hepatocellular carcinoma over a period of 10 to 20 years. In the US, it is estimated that infection with HCV accounts for 50,000 new cases of acute hepatitis in the United States each year (NIH Consensus Development Conference Statement on Management of Hepatitis C March 1997). The prevalence of HCV in the United States is estimated at 1.8% and the CDC places the number of chronically infected Americans at approximately 4.5 million people. The CDC also estimates that up to 10,000 deaths per year are caused by chronic HCV infection.

Numerous well controlled clinical trials using interferon (IFN-alpha) in the treatment of chronic HCV infection have demonstrated that treatment three times a week results in lowering of serum ALT values in approximately 50% (40% - 70%) of patients by the end of 6 months of therapy (Davis *et al.*, 1989, *New England Journal of Medicine*, 321, 1501-1506; Marcellin *et al.*, 1991, *Hepatology*, 13, 393-397; Tong *et al.*, 1997, *Hepatology*, 26, 747-754; Tong *et al.*, 1997, *Hepatology*, 26, 1640-1645). However, following cessation of interferon treatment, approximately 50% of the responding patients relapsed, resulting in a "durable" response rate as assessed by normalization of serum ALT concentrations of approximately 20 - 25%.

Direct measurement of HCV RNA is possible through use of either the branched-DNA or Reverse Transcriptase Polymerase Chain Reaction (RT-PCR) analysis. In general, RT-PCR methodology is more sensitive and leads to a more accurate assessment of the clinical course (Tong *et al.*, *supra*). Studies that have examined six months of type 1 interferon therapy using changes in HCV RNA values as a clinical endpoint have demonstrated that up to 35% of patients have a loss of HCV RNA by the end of therapy (Marcellin *et al.*, *supra*). However, as with the ALT endpoint, about 50% of the patients relapse within six months following cessation of therapy, resulting in a durable virologic response of only 12% (Marcellin *et al.*, *supra*). Studies that have examined 48 weeks of therapy have demonstrated that the sustained virological response is up to 25% (NIH

consensus statement: 1997). Thus, standard of care for treatment of chronic HCV infection with type 1 interferon is now 48 weeks of therapy using changes in HCV RNA concentrations as the primary assessment of efficacy (Hoofnagle *et al.*, 1997, *New England Journal of Medicine*, 336, 347-356).

5 Side effects resulting from treatment with type 1 interferons can be divided into four general categories, which include: (1) Influenza-like symptoms; (2) Neuropsychiatric; (3) Laboratory abnormalities; and (4) Miscellaneous (Dusheiko *et al.*, 1994, *Journal of Viral Hepatitis*, 1, 3-5). Examples of influenza-like symptoms include fatigue, fever, myalgia, malaise, appetite loss, tachycardia, rigors, headache, and
10 arthralgias. The influenza-like symptoms are usually short-lived and tend to abate after the first four weeks of dosing (Dushieko *et al.*, *supra*). Neuropsychiatric side effects include irritability, apathy, mood changes, insomnia, cognitive changes, and depression. The most important of these neuropsychiatric side effects is depression and patients who have a history of depression should not be given type 1 interferon. Laboratory
15 abnormalities include reduction in myeloid cells, including granulocytes, platelets and to a lesser extent red blood cells. These changes in blood cell counts rarely lead to any significant clinical sequelae (Dushieko *et al.*, *supra*). In addition, increases in triglyceride concentrations and elevations in serum alanine and aspartate aminotransferase concentration have been observed. Finally, thyroid abnormalities have
20 been reported. These thyroid abnormalities are usually reversible after cessation of interferon therapy and can be controlled with appropriate medication while on therapy. Miscellaneous side effects include nausea, diarrhea, abdominal and back pain, pruritus, alopecia, and rhinorrhea. In general, most side effects will abate after 4 to 8 weeks of therapy (Dushieko *et al.*, *supra*).

25 The use of small interfering nucleic acid molecules targeting HCV genes therefore provides a class of novel therapeutic agents that can be used in the treatment and diagnosis of HCV infection, liver failure, hepatocellular carcinoma, cirrhosis or any other disease or condition that responds to modulation of HCV genes.

Examples:

30 The following are non-limiting examples showing the selection, isolation, synthesis and activity of nucleic acids of the instant invention.

Example 1: Tandem synthesis of siNA constructs

Exemplary siNA molecules of the invention are synthesized in tandem using a cleavable linker, for example a succinyl-based linker. Tandem synthesis as described herein is followed by a one-step purification process that provides RNAi molecules in high yield. This approach is highly amenable to siNA synthesis in support of high throughput RNAi screening, and can be readily adapted to multi-column or multi-well synthesis platforms.

After completing a tandem synthesis of a siNA oligo and its complement in which the 5'-terminal dimethoxytrityl (5'-O-DMT) group remains intact (trityl on synthesis), the oligonucleotides are deprotected as described above. Following deprotection, the siNA sequence strands are allowed to spontaneously hybridize. This hybridization yields a duplex in which one strand has retained the 5'-O-DMT group while the complementary strand comprises a terminal 5'-hydroxyl. The newly formed duplex behaves as a single molecule during routine solid-phase extraction purification (Trityl-On purification) even though only one molecule has a dimethoxytrityl group. Because the strands form a stable duplex, this dimethoxytrityl group (or an equivalent group, such as other trityl groups or other hydrophobic moieties) is all that is required to purify the pair of oligos, for example by using a C18 cartridge.

Standard phosphoramidite synthesis chemistry is used up to the point of introducing a tandem linker, such as an inverted deoxy abasic succinate or glyceryl succinate linker (see **Figure 1**) or an equivalent cleavable linker. A non-limiting example of linker coupling conditions that can be used includes a hindered base such as diisopropylethylamine (DIPA) and/or DMAP in the presence of an activator reagent such as Bromotripyrrolidinophosphoniumhexafluorophosphate (PyBrOP). After the linker is coupled, standard synthesis chemistry is utilized to complete synthesis of the second sequence leaving the terminal the 5'-O-DMT intact. Following synthesis, the resulting oligonucleotide is deprotected according to the procedures described herein and quenched with a suitable buffer, for example with 50mM NaOAc or 1.5M $\text{NH}_4\text{H}_2\text{CO}_3$.

Purification of the siNA duplex can be readily accomplished using solid phase extraction, for example using a Waters C18 SepPak 1g cartridge conditioned with 1 column volume (CV) of acetonitrile, 2 CV H_2O , and 2 CV 50mM NaOAc. The sample

is loaded and then washed with 1 CV H₂O or 50mM NaOAc. Failure sequences are eluted with 1 CV 14% ACN (Aqueous with 50mM NaOAc and 50mM NaCl). The column is then washed, for example with 1 CV H₂O followed by on-column detritylation, for example by passing 1 CV of 1% aqueous trifluoroacetic acid (TFA) over the column, then adding a second CV of 1% aqueous TFA to the column and allowing to stand for approximately 10 minutes. The remaining TFA solution is removed and the column washed with H₂O followed by 1 CV 1M NaCl and additional H₂O. The siNA duplex product is then eluted, for example using 1 CV 20% aqueous CAN.

Figure 2 provides an example of MALDI-TOV mass spectrometry analysis of a purified siNA construct in which each peak corresponds to the calculated mass of an individual siNA strand of the siNA duplex. The same purified siNA provides three peaks when analyzed by capillary gel electrophoresis (CGE), one peak presumably corresponding to the duplex siNA, and two peaks presumably corresponding to the separate siNA sequence strands. Ion exchange HPLC analysis of the same siNA construct only shows a single peak. Testing of the purified siNA construct using a luciferase reporter assay described below demonstrated the same RNAi activity compared to siNA constructs generated from separately synthesized oligonucleotide sequence strands.

Example 2: Identification of potential siNA target sites in any RNA sequence

The sequence of an RNA target of interest, such as a viral or human mRNA transcript, is screened for target sites, for example by using a computer folding algorithm. In a non-limiting example, the sequence of a gene or RNA gene transcript derived from a database, such as Genbank, is used to generate siNA targets having complementarity to the target. Such sequences can be obtained from a database, or can be determined experimentally as known in the art. Target sites that are known, for example, those target sites determined to be effective target sites based on studies with other nucleic acid molecules, for example ribozymes or antisense, or those targets known to be associated with a disease or condition such as those sites containing mutations or deletions, can be used to design siNA molecules targeting those sites. Various parameters can be used to determine which sites are the most suitable target sites within the target RNA sequence. These parameters include but are not limited to secondary or

tertiary RNA structure, the nucleotide base composition of the target sequence, the degree of homology between various regions of the target sequence, or the relative position of the target sequence within the RNA transcript. Based on these determinations, any number of target sites within the RNA transcript can be chosen to screen siNA molecules for efficacy, for example by using *in vitro* RNA cleavage assays, cell culture, or animal models. In a non-limiting example, anywhere from 1 to 1000 target sites are chosen within the transcript based on the size of the siNA construct to be used. High throughput screening assays can be developed for screening siNA molecules using methods known in the art, such as with multi-well or multi-plate assays to determine efficient reduction in target gene expression.

Example 3: Selection of siNA molecule target sites in a RNA

The following non-limiting steps can be used to carry out the selection of siNAs targeting a given gene sequence or transcript.

1. The target sequence is parsed *in silico* into a list of all fragments or subsequences of a particular length, for example 23 nucleotide fragments, contained within the target sequence. This step is typically carried out using a custom Perl script, but commercial sequence analysis programs such as Oligo, MacVector, or the GCG Wisconsin Package can be employed as well.
2. In some instances the siNAs correspond to more than one target sequence; such would be the case for example in targeting different transcripts of the same gene, targeting different transcripts of more than one gene, or for targeting both the human gene and an animal homolog. In this case, a subsequence list of a particular length is generated for each of the targets, and then the lists are compared to find matching sequences in each list. The subsequences are then ranked according to the number of target sequences that contain the given subsequence; the goal is to find subsequences that are present in most or all of the target sequences. Alternately, the ranking can identify subsequences that are unique to a target sequence, such as a mutant target sequence. Such an approach would enable the use of siNA to target specifically the mutant sequence and not effect the expression of the normal sequence.

3. In some instances the siNA subsequences are absent in one or more sequences while present in the desired target sequence; such would be the case if the siNA targets a gene with a paralogous family member that is to remain untargeted. As in case 2 above, a subsequence list of a particular length is generated for each of the targets,
5 and then the lists are compared to find sequences that are present in the target gene but are absent in the untargeted paralog.
4. The ranked siNA subsequences can be further analyzed and ranked according to GC content. A preference can be given to sites containing 30-70% GC, with a further preference to sites containing 40-60% GC.
- 10 5. The ranked siNA subsequences can be further analyzed and ranked according to self-folding and internal hairpins. Weaker internal folds are preferred; strong hairpin structures are to be avoided.
6. The ranked siNA subsequences can be further analyzed and ranked according to whether they have runs of GGG or CCC in the sequence. GGG (or even more Gs) in
15 either strand can make oligonucleotide synthesis problematic and can potentially interfere with RNAi activity, so it is avoided whenever better sequences are available. CCC is searched in the target strand because that will place GGG in the antisense strand.
7. The ranked siNA subsequences can be further analyzed and ranked according to
20 whether they have the dinucleotide UU (uridine dinucleotide) on the 3'-end of the sequence, and/or AA on the 5'-end of the sequence (to yield 3' UU on the antisense sequence). These sequences allow one to design siNA molecules with terminal TT thymidine dinucleotides.
8. Four or five target sites are chosen from the ranked list of subsequences as described
25 above. For example, in subsequences having 23 nucleotides, the right 21 nucleotides of each chosen 23-mer subsequence are then designed and synthesized for the upper (sense) strand of the siNA duplex, while the reverse complement of the left 21 nucleotides of each chosen 23-mer subsequence are then designed and synthesized for the lower (antisense) strand of the siNA duplex (see Tables II and III). If
30 terminal TT residues are desired for the sequence (as described in paragraph 7), then

the two 3' terminal nucleotides of both the sense and antisense strands are replaced by TT prior to synthesizing the oligos.

9. The siNA molecules are screened in an *in vitro*, cell culture or animal model system to identify the most active siNA molecule or the most preferred target site within the target RNA sequence.

In an alternate approach, a pool of siNA constructs specific to a HCV target sequence is used to screen for target sites in cells expressing HCV RNA, such as the human hepatoma (Huh7) cells (see for example Randall *et al.*, 2003, *PNAS USA*, 100, 235-240). The general strategy used in this approach is shown in Figure 9. A non-limiting example of such is a pool comprising sequences having sense sequences comprising SEQ ID NOs. 1-696, 1393-1413, 1417-1419, 1421-1427, 1449-1455, 1477, 1481, 1485, 1487, 1494-1496, 1499, 1501-1512, 1549, 1553, 1558-1569, 1582-1593, 1617, 1619, 1621, 1623, and 1625 and antisense sequences comprising SEQ ID NOs. 697-1392, 1414, 1420, 1428-1434, 1456-1462, 1479, 1483, 1489-1491, 1493, 1497-1498, 1500, 1513-1524, 1551, 1556, 1570-1581, 1618, 1620, 1622, 1624, 1626, and 1627 respectively. Cells expressing HCV (e.g., Huh7 cells) are transfected with the pool of siNA constructs and cells that demonstrate a phenotype associated with HCV inhibition are sorted. The pool of siNA constructs can be expressed from transcription cassettes inserted into appropriate vectors (see for example Figure 7 and Figure 8). The siNA from cells demonstrating a positive phenotypic change (e.g., decreased proliferation, decreased HCV mRNA levels or decreased HCV protein expression), are sequenced to determine the most suitable target site(s) within the target HCV RNA sequence.

Example 4: HCV targeted siNA design

siNA target sites were chosen by analyzing sequences of the HCV RNA target and optionally prioritizing the target sites on the basis of folding (structure of any given sequence analyzed to determine siNA accessibility to the target), by using a library of siNA molecules as described in Example 3, or alternately by using an *in vitro* siNA system as described in Example 6 herein. siNA molecules were designed that could bind each target and are optionally individually analyzed by computer folding to assess whether the siNA molecule can interact with the target sequence. Varying the length of

the siNA molecules can be chosen to optimize activity. Generally, a sufficient number of complementary nucleotide bases are chosen to bind to, or otherwise interact with, the target RNA, but the degree of complementarity can be modulated to accommodate siNA duplexes or varying length or base composition. By using such methodologies, siNA molecules can be designed to target sites within any known RNA sequence, for example those RNA sequences corresponding to the any gene transcript.

Chemically modified siNA constructs are designed to provide nuclease stability for systemic administration in vivo and/or improved pharmacokinetic, localization, and delivery properties while preserving the ability to mediate RNAi activity. Chemical modifications as described herein are introduced synthetically using synthetic methods described herein and those generally known in the art. The synthetic siNA constructs are then assayed for nuclease stability in serum and/or cellular/tissue extracts (e.g. liver extracts). The synthetic siNA constructs are also tested in parallel for RNAi activity using an appropriate assay, such as a luciferase reporter assay as described herein or another suitable assay that can quantify RNAi activity. Synthetic siNA constructs that possess both nuclease stability and RNAi activity can be further modified and re-evaluated in stability and activity assays. The chemical modifications of the stabilized active siNA constructs can then be applied to any siNA sequence targeting any chosen RNA and used, for example, in target screening assays to pick lead siNA compounds for therapeutic development (see for example **Figure 11**).

Example 5: Chemical Synthesis and Purification of siNA

siNA molecules can be designed to interact with various sites in the RNA message, for example, target sequences within the RNA sequences described herein. The sequence of one strand of the siNA molecule(s) is complementary to the target site sequences described above. The siNA molecules can be chemically synthesized using methods described herein. Inactive siNA molecules that are used as control sequences can be synthesized by scrambling the sequence of the siNA molecules such that it is not complementary to the target sequence. Generally, siNA constructs can be synthesized using solid phase oligonucleotide synthesis methods as described herein (see for example Usman *et al.*, US Patent Nos. 5,804,683; 5,831,071; 5,998,203; 6,117,657; 6,353,098; 6,362,323; 6,437,117; 6,469,158; Scaringe *et al.*, US Patent Nos. 6,111,086; 6,008,400; 6,111,086 all incorporated by reference herein in their entirety).

In a non-limiting example, RNA oligonucleotides are synthesized in a stepwise fashion using the phosphoramidite chemistry as is known in the art. Standard phosphoramidite chemistry involves the use of nucleosides comprising any of 5'-O-dimethoxytrityl, 2'-O-tert-butyldimethylsilyl, 3'-O-2-Cyanoethyl N,N-diisopropylphosphoroamidite groups, and exocyclic amine protecting groups (e.g. N6-benzoyl adenosine, N4 acetyl cytidine, and N2-isobutyryl guanosine). Alternately, 2'-O-Silyl Ethers can be used in conjunction with acid-labile 2'-O-orthoester protecting groups in the synthesis of RNA as described by Scaringe *supra*. Differing 2' chemistries can require different protecting groups, for example 2'-deoxy-2'-amino nucleosides can utilize N-phthaloyl protection as described by Usman *et al.*, US Patent 5,631,360, incorporated by reference herein in its entirety).

During solid phase synthesis, each nucleotide is added sequentially (3'- to 5'-direction) to the solid support-bound oligonucleotide. The first nucleoside at the 3'-end of the chain is covalently attached to a solid support (e.g., controlled pore glass or polystyrene) using various linkers. The nucleotide precursor, a ribonucleoside phosphoramidite, and activator are combined resulting in the coupling of the second nucleoside phosphoramidite onto the 5'-end of the first nucleoside. The support is then washed and any unreacted 5'-hydroxyl groups are capped with a capping reagent such as acetic anhydride to yield inactive 5'-acetyl moieties. The trivalent phosphorus linkage is then oxidized to a more stable phosphate linkage. At the end of the nucleotide addition cycle, the 5'-O-protecting group is cleaved under suitable conditions (e.g., acidic conditions for trityl-based groups and Fluoride for silyl-based groups). The cycle is repeated for each subsequent nucleotide.

Modification of synthesis conditions can be used to optimize coupling efficiency, for example by using differing coupling times, differing reagent/phosphoramidite concentrations, differing contact times, differing solid supports and solid support linker chemistries depending on the particular chemical composition of the siNA to be synthesized. Deprotection and purification of the siNA can be performed as is generally described in Usman *et al.*, US 5,831,071, US 6,353,098, US 6,437,117, Bellon *et al.*, US 6,054,576, US 6,162,909, US 6,303,773, and Scaringe *supra*, all of which are incorporated by reference herein in their entireties. Additionally, deprotection conditions can be modified to provide the best possible yield and purity of siNA constructs. For

example, applicant has observed that oligonucleotides comprising 2'-deoxy-2'-fluoro nucleotides can degrade under inappropriate deprotection conditions. Such oligonucleotides are deprotected using aqueous methylamine at about 35°C for 30 minutes. If the 2'-deoxy-2'-fluoro containing oligonucleotide also comprises
5 ribonucleotides, after deprotection with aqueous methylamine at about 35°C for 30 minutes, TEA-HF is added and the reaction maintained at about 65°C for an additional 15 minutes.

Example 6: RNAi *in vitro* assay to assess siNA activity

An *in vitro* assay that recapitulates RNAi in a cell-free system is used to evaluate
10 siNA constructs targeting HCV RNA targets. The assay comprises the system described by Tuschl *et al.*, 1999, *Genes and Development*, 13, 3191-3197 and Zamore *et al.*, 2000, *Cell*, 101, 25-33 adapted for use with HCV target RNA. A *Drosophila* extract derived from syncytial blastoderm is used to reconstitute RNAi activity *in vitro*. Target RNA is generated via *in vitro* transcription from an appropriate HCV expressing plasmid using
15 T7 RNA polymerase or via chemical synthesis as described herein. Sense and antisense siNA strands (for example 20 uM each) are annealed by incubation in buffer (such as 100 mM potassium acetate, 30 mM HEPES-KOH, pH 7.4, 2 mM magnesium acetate) for 1 min. at 90°C followed by 1 hour at 37°C, then diluted in lysis buffer (for example 100 mM potassium acetate, 30 mM HEPES-KOH at pH 7.4, 2mM magnesium acetate).
20 Annealing can be monitored by gel electrophoresis on an agarose gel in TBE buffer and stained with ethidium bromide. The *Drosophila* lysate is prepared using zero to two-hour-old embryos from Oregon R flies collected on yeasted molasses agar that are dechorionated and lysed. The lysate is centrifuged and the supernatant isolated. The assay comprises a reaction mixture containing 50% lysate [vol/vol], RNA (10-50 pM
25 final concentration), and 10% [vol/vol] lysis buffer containing siNA (10 nM final concentration). The reaction mixture also contains 10 mM creatine phosphate, 10 ug/ml creatine phosphokinase, 100 uM GTP, 100 uM UTP, 100 uM CTP, 500 uM ATP, 5 mM DTT, 0.1 U/uL RNasin (Promega), and 100 uM of each amino acid. The final concentration of potassium acetate is adjusted to 100 mM. The reactions are pre-
30 assembled on ice and preincubated at 25° C for 10 minutes before adding RNA, then incubated at 25° C for an additional 60 minutes. Reactions are quenched with 4 volumes of 1.25 x Passive Lysis Buffer (Promega). Target RNA cleavage is assayed by RT-PCR

analysis or other methods known in the art and are compared to control reactions in which siNA is omitted from the reaction.

Alternately, internally-labeled target RNA for the assay is prepared by *in vitro* transcription in the presence of [α - 32 P] CTP, passed over a G 50 Sephadex column
5 by spin chromatography and used as target RNA without further purification. Optionally, target RNA is 5'- 32 P-end labeled using T4 polynucleotide kinase enzyme. Assays are performed as described above and target RNA and the specific RNA cleavage products generated by RNAi are visualized on an autoradiograph of a gel. The percentage of cleavage is determined by Phosphor Imager[®] quantitation of bands
10 representing intact control RNA or RNA from control reactions without siNA and the cleavage products generated by the assay.

In one embodiment, this assay is used to determine target sites the HCV RNA target for siNA mediated RNAi cleavage, wherein a plurality of siNA constructs are screened for RNAi mediated cleavage of the HCV RNA target, for example, by
15 analyzing the assay reaction by electrophoresis of labeled target RNA, or by northern blotting, as well as by other methodology well known in the art.

Example 7: Nucleic acid inhibition of HCV target RNA *in vivo*

siNA molecules targeted to the huma HCV RNA are designed and synthesized as described above. These nucleic acid molecules can be tested for cleavage activity *in*
20 *vivo*, for example, using the following procedure. The target sequences and the nucleotide location within the HCV RNA are given in Table II and III.

Two formats are used to test the efficacy of siNAs targeting HCV. First, the reagents are tested in cell culture using, for example, Huh7 cells (see, for example, Randall *et al.*, 2003, *PNAS USA*, 100, 235-240) to determine the extent of RNA and
25 protein inhibition. siNA reagents (*e.g.*; see Tables II and III) are selected against the HCV target as described herein. RNA inhibition is measured after delivery of these reagents by a suitable transfection agent to, for example, Huh7 cells. Relative amounts of target RNA are measured versus actin using real-time PCR monitoring of amplification (*eg.*, ABI 7700 Taqman[®]). A comparison is made to a mixture of
30 oligonucleotide sequences made to unrelated targets or to a randomized siNA control

with the same overall length and chemistry, but randomly substituted at each position. Primary and secondary lead reagents are chosen for the target and optimization performed. After an optimal transfection agent concentration is chosen, a RNA time-course of inhibition is performed with the lead siNA molecule.

5 In addition, a cell-plating format can be used to determine RNA inhibition. A non-limiting example of a multiple target screen to assay siNA mediated inhibition of HCV RNA is shown in Figure 18. siNA constructs (Table III) were transfected at 25 nM into Huh7 cells and HCV RNA quantitated compared to untreated cells ("cells" column in the figure) and cells transfected with lipofectamine ("LFA2K" column in the figure). As
10 shown in Figure 18, several siNA constructs show significant inhibition of HCV RNA expression in the Huh7 replicon system. This system is described in Rice *et al.*, US 5,874,565 and US 6,127,116, both incorporated by reference herein.

Delivery of siNA to Cells

Huh7b cells stably transfected with the HCV subgenomic replicon Clone A or
15 Ava.5 are seeded, for example, at 8.5×10^3 cells per well of a 96-well plate in DMEM(Gibco) the day before transfection. siNA (final concentration, for example 25nM) and cationic lipid Lipofectamine2000 (e.g., final concentration 0.5ul/well) are complexed in Optimem (Gibco) at 37°C for 20 minutes in polypropylene microtubes. Following vortexing, the complexed siNA is added to each well and incubated for 24-72
20 hrs.

Taqman quantification of mRNA

Total RNA is prepared from cells following siNA delivery, for example, using Ambion Rnaqueous 4-PCR purification kit for large scale extractions, or Ambion Rnaqueous-96 purification kit for 96-well assays. For Taqman analysis, dual-labeled
25 probes are synthesized with, for example, the reporter dyes FAM or VIC covalently linked at the 5'-end and the quencher dye TAMARA conjugated to the 3'-end. One-step RT-PCR amplifications are performed on, for example, an ABI PRISM 7700 Sequence detector using 50uL reactions consisting of 10uL total RNA, 100nM forward primer, 100nM reverse primer, 100nM probe, 1X TaqMan PCR reaction buffer (PE-Applied Biosystems), 5.5mM MgCl₂, 100uM each dATP, dCTP, dGTP and dTTP, 0.2U RNase
30

Inhibitor (Promega), 0.025U AmpliTaq Gold (PE-Applied Biosystems) and 0.2U M-MLV Reverse Transcriptase (Promega). The thermal cycling conditions can consist of 30 min at 48°C, 10min at 95°C, followed by 40 cycles of 15sec at 95°C and 1 min at 60°C. Quantitation of target mRNA level is determined relative to standards generated from serially diluted total cellular RNA (300, 100, 30, 10 ng/rxn) and normalizing to, for example, 36B4 mRNA in either parallel or same tube TaqMan reactions. For HCV Replicon mRNA quantitation, PCR primers and probe specific for the neomycin gene were used:

neo-forward primer, 5'-CCGGCTACCTGCCCCATTC-3'; (SEQ ID NO: 1628)
 10 neo-reverse primer, 5'-CCAGATCATCCTGATCGACAAG-3'; (SEQ ID NO: 1629)
 neo-probe, 5'FAM-ACATCGCATCGAGCGAGCACGTAC-TAMARA3'; (SEQ ID NO: 1630)
 For normalization, 36B4 PCR primers and probe were used:
 36B4-forward primer, 5'-TCTATCATCAACGGGTACAAACGA-3'; (SEQ ID NO:
 15 1631)
 36B4 reverse primer, 5'-CTTTTCAGCAAGTGGGAAGGTG-3'; (SEQ ID NO: 1632)
 36B4 probe, 5'VIC-CCTGGCCTTGTCTGTGGAGACGGATTA-TAMARA3'; (SEQ ID NO: 1633)

20 Western blotting

Nuclear extracts can be prepared using a standard micro preparation technique (see for example Andrews and Faller, 1991, *Nucleic Acids Research*, 19, 2499). Protein extracts from supernatants are prepared, for example using TCA precipitation. An equal volume of 20% TCA is added to the cell supernatant, incubated on ice for 1 hour and
 25 pelleted by centrifugation for 5 minutes. Pellets are washed in acetone, dried and resuspended in water. Cellular protein extracts are run on a 10% Bis-Tris NuPage (nuclear extracts) or 4-12% Tris-Glycine (supernatant extracts) polyacrylamide gel and transferred onto nitro-cellulose membranes. Non-specific binding can be blocked by incubation, for example, with 5% non-fat milk for 1 hour followed by primary antibody
 30 for 16 hour at 4°C. Following washes, the secondary antibody is applied, for example (1:10,000 dilution) for 1 hour at room temperature and the signal detected with SuperSignal reagent (Pierce).

Example 8: Models useful to evaluate the down-regulation of HCV gene expression*Cell Culture*

Although there have been reports of replication of HCV in cell culture (see below), these systems are difficult to reproduce and have proven unreliable. Therefore, as was
5 the case for development of other anti-HCV therapeutics, such as interferon and ribavirin, after demonstration of safety in animal studies applicant can proceed directly into a clinical feasibility study.

Several recent reports have documented *in vitro* growth of HCV in human cell lines (Mizutani *et al.*, Biochem Biophys Res Commun 1996 227(3):822-826; Tagawa *et al.*,
10 *Journal of Gastroenterology and Hepatology* 1995 10(5):523-527; Cribier *et al.*, *Journal of General Virology* 76(10):2485-2491; Seipp *et al.*, *Journal of General Virology* 1997 78(10):2467-2478; Iacovacci *et al.*, *Research Virology* 1997 148(2):147-151; Iacovacci *et al.*, *Hepatology* 1997 26(5) 1328-1337; Ito *et al.*, *Journal of General Virology* 1996 77(5):1043-1054; Nakajima *et al.*, *Journal of Virology* 1996 70(5):3325-
15 3329; Mizutani *et al.*, *Journal of Virology* 1996 70(10):7219-7223; Valli *et al.*, *Res Virol* 1995 146(4): 285-288; Kato *et al.*, *Biochem Biophys Res Comm* 1995 206(3):863-869). Replication of HCV has been reported in both T and B cell lines, as well as cell lines derived from human hepatocytes. Detection of low level replication was documented using either RT-PCR based assays or the b-DNA assay. It is important to note that the
20 most recent publications regarding HCV cell cultures document replication for up to 6-months. However, the level of HCV replication observed in these cell lines has not been robust enough for screening of antiviral compounds.

In addition to cell lines that can be infected with HCV, several groups have reported the successful transformation of cell lines with cDNA clones of full-length or
25 partial HCV genomes (Harada *et al.*, *Journal of General Virology*, 1995, 76(5):1215-1221; Haramatsu *et al.*, *Journal of Viral Hepatitis* 1997 4S(1):61-67; Dash *et al.*, *American Journal of Pathology* 1997 151(2):363-373; Mizuno *et al.*, *Gastroenterology* 1995 109(6):1933-40; Yoo *et al.*, *Journal Of Virology* 1995 69(1):32-38).

The recent development of subgenomic HCV RNA replicons capable of
30 successfully replicating in the human hepatoma cell line, Huh7, represents a significant

advance toward a dependable cell culture model. These replicons contain the neomycin gene upstream of the HCV nonstructural genes allowing for the selection of replicative RNAs in Huh7 cells. Initially, RNA replication was detected at a low frequency (Lohmann *et al.* Science 1999 285: 110-113) but the identification of replicons with cell-
5 adaptive mutations in the NS5A region has improved the efficiency of replication 10,000-fold (Blight *et al.* Science 2000 290:1972-1975). Steps in the HCV life cycle, such as translation, protein processing, and RNA replication are recapitulated in the subgenomic replicon systems, but early events (viral attachment and uncoating) and viral assembly is absent. Inclusion of the structural genes of HCV within the replicons results
10 in the production of HCV core and envelope proteins, but virus assembly does not occur (Pietschmann *et al.* Journal of Virology 2002 76: 4008-4021). Such replicon systems have been used to study siRNA mediated inhibition of HCV RNA, see for example, Randall *et al.*, 2003, *PNAS USA*, 100, 235-240.

In several cell culture systems, cationic lipids have been shown to enhance the
15 bioavailability of oligonucleotides to cells in culture (Bennet, *et al.*, 1992, *Mol. Pharmacology*, 41, 1023-1033). In one embodiment, siNA molecules of the invention are complexed with cationic lipids for cell culture experiments. siNA and cationic lipid mixtures are prepared in serum-free DMEM immediately prior to addition to the cells. DMEM plus additives are warmed to room temperature (about 20-25°C) and cationic
20 lipid is added to the final desired concentration and the solution is vortexed briefly. siNA molecules are added to the final desired concentration and the solution is again vortexed briefly and incubated for 10 minutes at room temperature. In dose response experiments, the RNA/lipid complex is serially diluted into DMEM following the 10 minute incubation.

25 *Animal Models*

Evaluating the efficacy of anti-HCV agents in animal models is an important prerequisite to human clinical trials. The best characterized animal system for HCV infection is the chimpanzee. Moreover, the chronic hepatitis that results from HCV infection in chimpanzees and humans is very similar. Although clinically relevant, the
30 chimpanzee model suffers from several practical impediments that make use of this model difficult. These include high cost, long incubation requirements and lack of

sufficient quantities of animals. Due to these factors, a number of groups have attempted to develop rodent models of chronic hepatitis C infection. While direct infection has not been possible, several groups have reported on the stable transfection of either portions or entire HCV genomes into rodents (Yamamoto *et al.*, Hepatology 1995 22(3): 847-855; Galun *et al.*, Journal of Infectious Disease 1995 172(1):25-30; Koike *et al.*, Journal of general Virology 1995 76(12):3031-3038; Pasquinelli *et al.*, Hepatology 1997 25(3): 719-727; Hayashi *et al.*, Princess Takamatsu Symp 1995 25:1430149; Mariya *et al.*, Journal of General Virology 1997 78(7) 1527-1531; Takehara *et al.*, Hepatology 1995 21(3):746-751; Kawamura *et al.*, Hepatology 1997 25(4): 1014-1021). In addition, transplantation of HCV infected human liver into immunocompromised mice results in prolonged detection of HCV RNA in the animal's blood.

A method for expressing hepatitis C virus in an *in vivo* animal model has been developed (Vierling, International PCT Publication No. WO 99/16307). Viable, HCV infected human hepatocytes are transplanted into a liver parenchyma of a scid/scid mouse host. The scid/scid mouse host is then maintained in a viable state, whereby viable, morphologically intact human hepatocytes persist in the donor tissue and hepatitis C virus is replicated in the persisting human hepatocytes. This model provides an effective means for the study of HCV inhibition by enzymatic nucleic acids *in vivo*.

Example 9: RNAi mediated inhibition of HCV RNA expression

siNA constructs (e.g., siNA constructs shown in Table III) are tested for efficacy in reducing HCV RNA expression in, for example, Huh7 cells (see, for example, Randall *et al.*, 2003, *PNAS USA*, 100, 235-240). Cells are plated approximately 24h before transfection in 96-well plates at 5,000-7,500 cells/well, 100 μ l/well, such that at the time of transfection cells are 70-90% confluent. For transfection, annealed siNAs are mixed with the transfection reagent (Lipofectamine 2000, Invitrogen) in a volume of 50 μ l/well and incubated for 20 minutes at room temperature. The siNA transfection mixtures are added to cells to give a final siNA concentration of 25 nM in a volume of 150 μ l. Each siNA transfection mixture is added to 3 wells for triplicate siNA treatments. Cells are incubated at 37° for 24h in the continued presence of the siNA transfection mixture. At 24h, RNA is prepared from each well of treated cells. The supernatants with the transfection mixtures are first removed and discarded, then the cells are lysed and RNA

prepared from each well. Target gene expression following treatment is evaluated by RT-PCR for the target gene and for a control gene (36B4, an RNA polymerase subunit) for normalization. The triplicate data is averaged and the standard deviations determined for each treatment. Normalized data are graphed and the percent reduction of target mRNA by active siNAs in comparison to their respective inverted control siNAs is determined.

In a non-limiting example, a siNA construct comprising ribonucleotides and 3'-terminal dithymidine caps is assayed along with a chemically modified siNA construct comprising 2'-deoxy-2'-fluoro pyrimidine nucleotides and purine ribonucleotides in which the sense strand of the siNA is further modified with 5' and 3'-terminal inverted deoxyabasic caps and the antisense strand comprises a 3'-terminal phosphorothioate internucleotide linkage. Additional stabilization chemistries as described in Table IV are similarly assayed for activity. These siNA constructs are compared to appropriate matched chemistry inverted controls. In addition, the siNA constructs are also compared to untreated cells, cells transfected with lipid and scrambled siNA constructs, and cells transfected with lipid alone (transfection control).

Example 10: siNA Inhibition of a chimeric HCV/Poliovirus in HeLa Cells

Inhibition of a chimeric HCV/Poliovirus was investigated using 21 nucleotide siNA duplexes in HeLa cells. Seven siNA were designed that target three regions in the highly conserved 5' untranslated region (UTR) of HCV RNA. The siNAs were screened in two cell culture systems dependent upon the 5'-UTR of HCV; one requires translation of an HCV/luciferase gene, while the other involves replication of a chimeric HCV/poliovirus (PV) (see Blatt *et al.*, USSN 09/740,332, filed December 18, 2000, incorporated by reference herein). Transfection for the HCV/PV system was performed in HeLa cells (grown in DMEM supplemented with sodium pyruvate and 100mM HEPES with 5% FBS) using either cationic lipid NC168 or LFA2K, with an siNA concentration of 10nM or 25nM. HeLa cells were inoculated with HCV/PV virus at an moi=.01 pfu/cell for 30 minutes in serum-free media. The inoculum was removed and 80μL media was added, with 20μL of transfection complex added to each well. The cells and supernatants were frozen at 20-24 hours post transfection. Each plate underwent 3 freeze-thaw cycles and the supernatant was collected. The supernatant was

titered on HeLa cells for 3 days, then stained and counted. The results shown in Figures 14-17 are reported as pfu/ml $\times 10^5$.

Two siNAs (29579/29586 and 29578/2958) targeting the same region (shifted by one nucleotide) are active in both systems (see Figure 12). For example, a >85%
5 reduction in HCVPV replication was observed in siNA-treated cells compared to an inverse siNA control 29593/29600 (Figure 12) with an $IC_{50} = \sim 2.5$ nM (Figure 13). To develop nuclease-resistant siNA for in vivo applications, siNAs can be modified to contain stabilizing chemical modifications. Such modifications include phosphorothioate linkages (P=S), 2'-O-methyl nucleotides, 2'-fluoro (F) nucleotides, 2'-deoxy
10 nucleotides, universal base nucleotides, 5' and/or 3' end modifications and a variety of other nucleotide and non-nucleotide modifications, such as those described herein, in one or both siNA strands. Using this systematic approach, active siNA molecules have been identified that are substantially more resistant to nucleases. Several of these constructs were tested in the HCV/poliovirus chimera system, demonstrating significant reduction
15 in viral replication (see Figures 14-17). siNA constructs shown in Figures 14-17 are referred to by RPI#s that are cross referenced to Table III. siNA activity is compared to relevant controls (untreated cells, scrambled/inactive control sequences, or transfection controls). Figure 14 shows the inhibition of HCV RNA in the HCV/poliovirus chimera system using chemically modified siNA construct 30051/30053, which construct has
20 inverted deoxy abasic nucleotides at the 3' and 5' ends, several phosphorothioate linkages, and 5-nitroindole nucleotides. Figure 15 shows the inhibition of HCV RNA in the HCV/poliovirus chimera system using chemically modified siNA construct 30055/30057, which construct has inverted deoxy abasic nucleotides at the 3' and 5' ends, several phosphorothioate linkages, and 5-nitroindole nucleotides. Figures 16 and
25 17 show the inhibition of HCV RNA in the HCV/poliovirus chimera system using unmodified siNA construct (29586/29579) and chemically modified siNA constructs 30417/30419, 30417/30420, 30418/30419, and combinations thereof at 10 nM and 25 nM siNA, respectively. As shown in Figures 14-17, siNA constructs of the invention provide potent inhibition of HCV RNA in the HCV/poliovirus chimera system. As such,
30 siNA constructs, including chemically modified, nuclease resistant siNA molecules, represent an important class of therapeutic agents for treating chronic HCV infection.

Example 11: Indications

The present body of knowledge in HCV research indicates the need for methods to assay HCV activity and for compounds that can regulate HCV expression for research, diagnostic, and therapeutic use. As described herein, the nucleic acid molecules of the present invention can be used in assays to diagnose disease state related of HCV levels.

5 In addition, the nucleic acid molecules can be used to treat disease state related to HCV levels.

Particular degenerative and disease states that can be associated with HCV expression modulation include, but are not limited to, HCV infection, liver failure, hepatocellular carcinoma, cirrhosis, and/or other disease states associated with HCV
10 infection.

Example 12: Interferons

Interferons represent a non-limiting example of a class of compounds that can be used in conjunction with the siNA molecules of the invention for treating the diseases and/or conditions described herein. Type I interferons (IFN) are a class of natural
15 cytokines that includes a family of greater than 25 IFN- α (Pesta, 1986, *Methods Enzymol.* 119, 3-14) as well as IFN- β , and IFN- ω . Although evolutionarily derived from the same gene (Diaz *et al.*, 1994, *Genomics* 22, 540-552), there are many differences in the primary sequence of these molecules, implying an evolutionary divergence in biologic activity. All type I IFN share a common pattern of biologic effects that begin
20 with binding of the IFN to the cell surface receptor (Pfeffer & Strulovici, 1992, *Transmembrane secondary messengers for IFN- α/β* . In: *Interferon. Principles and Medical Applications.*, S. Baron, D.H. Coopenhaver, F. Dianzani, W.R. Fleischmann Jr., T.K. Hughes Jr., G.R. Kimpel, D.W. Niesel, G.J. Stanton, and S.K. Tying, eds. 151-160). Binding is followed by activation of tyrosine kinases, including the Janus tyrosine
25 kinases and the STAT proteins, which leads to the production of several IFN-stimulated gene products (Johnson *et al.*, 1994, *Sci. Am.* 270, 68-75). The IFN-stimulated gene products are responsible for the pleotropic biologic effects of type I IFN, including antiviral, antiproliferative, and immunomodulatory effects, cytokine induction, and HLA class I and class II regulation (Pestka *et al.*, 1987, *Annu. Rev. Biochem* 56, 727).
30 Examples of IFN-stimulated gene products include 2-5-oligoadenylate synthetase (2-5 OAS), β_2 -microglobulin, neopterin, p68 kinases, and the Mx protein (Chebath & Revel,

1992, The 2-5 A system: 2-5 A synthetase, isospecies and functions. In: *Interferon. Principles and Medical Applications*, S. Baron, D.H. Coopenhaver, F. Dianzani, W.R. Jr. Fleischmann, T.K. Jr Hughes, G.R. Kimpel, D.W. Niesel, G.J. Stanton, and S.K. Tying, eds., pp. 225-236; Samuel, 1992, The RNA-dependent P1/eIF-2 α protein kinase. In: 5 *Interferon. Principles and Medical Applications*. S. Baron, D.H. Coopenhaver, F. Dianzani, W.R. Fleischmann Jr., T.K. Hughes Jr., G.R. Kimpel, D.W. Niesel, G.H. Stanton, and S.K. Tying, eds. 237-250; Horisberger, 1992, MX protein: function and Mechanism of Action. In: *Interferon. Principles and Medical Applications*. S. Baron, D.H. Coopenhaver, F. Dianzani, W.R. Fleischmann Jr., T.K. Hughes Jr., G.R. Kimpel, 10 D.W. Niesel, G.H. Stanton, and S.K. Tying, eds. 215-224). Although all type I IFN have similar biologic effects, not all the activities are shared by each type I IFN, and in many cases, the extent of activity varies quite substantially for each IFN subtype (Fish *et al.*, 1989, *J. Interferon Res.* 9, 97-114; Ozes *et al.*, 1992, *J. Interferon Res.* 12, 55-59). More specifically, investigations into the properties of different subtypes of IFN- α and 15 molecular hybrids of IFN- α have shown differences in pharmacologic properties (Rubinstein, 1987, *J. Interferon Res.* 7, 545-551). These pharmacologic differences can arise from as few as three amino acid residue changes (Lee *et al.*, 1982, *Cancer Res.* 42, 1312-1316).

Eighty-five to 166 amino acids are conserved in the known IFN- α subtypes. 20 Excluding the IFN- α pseudogenes, there are approximately 25 known distinct IFN- α subtypes. Pairwise comparisons of these nonallelic subtypes show primary sequence differences ranging from 2% to 23%. In addition to the naturally occurring IFNs, a non-natural recombinant type I interferon known as consensus interferon (CIFN) has been synthesized as a therapeutic compound (Tong *et al.*, 1997, *Hepatology* 26, 747-754).

25 Interferon is currently in use for at least 12 different indications, including infectious and autoimmune diseases and cancer (Borden, 1992, *N. Engl. J. Med.* 326, 1491-1492). For autoimmune diseases, IFN has been utilized for treatment of rheumatoid arthritis, multiple sclerosis, and Crohn's disease. For treatment of cancer, IFN has been used alone or in combination with a number of different compounds. 30 Specific types of cancers for which IFN has been used include squamous cell carcinomas, melanomas, hypernephromas, hemangiomas, hairy cell leukemia, and Kaposi's sarcoma. In the treatment of infectious diseases, IFNs increase the phagocytic

activity of macrophages and cytotoxicity of lymphocytes and inhibits the propagation of cellular pathogens. Specific indications for which IFN has been used as treatment include hepatitis B, human papillomavirus types 6 and 11 (i.e. genital warts) (Leventhal *et al.*, 1991, *N Engl J Med* 325, 613-617), chronic granulomatous disease, and hepatitis C virus.

Numerous well controlled clinical trials using IFN-alpha in the treatment of chronic HCV infection have demonstrated that treatment three times a week results in lowering of serum ALT values in approximately 50% (range 40% to 70%) of patients by the end of 6 months of therapy (Davis *et al.*, 1989, *N. Engl. J. Med.* 321, 1501-1506; Marcellin *et al.*, 1991, *Hepatology* 13, 393-397; Tong *et al.*, 1997, *Hepatology* 26, 747-754; Tong *et al.*, *Hepatology* 26, 1640-1645). However, following cessation of interferon treatment, approximately 50% of the responding patients relapsed, resulting in a "durable" response rate as assessed by normalization of serum ALT concentrations of approximately 20 to 25%. In addition, studies that have examined six months of type 1 interferon therapy using changes in HCV RNA values as a clinical endpoint have demonstrated that up to 35% of patients will have a loss of HCV RNA by the end of therapy (Tong *et al.*, 1997, *supra*). However, as with the ALT endpoint, about 50% of the patients relapse six months following cessation of therapy resulting in a durable virologic response of only 12% (23). Studies that have examined 48 weeks of therapy have demonstrated that the sustained virological response is up to 25%.

Pegylated interferons, i.e., interferons conjugated with polyethylene glycol (PEG), have demonstrated improved characteristics over interferon. Advantages incurred by PEG conjugation can include an improved pharmacokinetic profile compared to interferons lacking PEG, thus imparting more convenient dosing regimes, improved tolerance, and improved antiviral efficacy. Such improvements have been demonstrated in clinical studies of both polyethylene glycol interferon alfa-2a (PEGASYS, Roche) and polyethylene glycol interferon alfa-2b (VIRAIFERON PEG, PEG-INTRON, Enzon/Schering Plough).

siNA molecules in combination with interferons and polyethylene glycol interferons have the potential to improve the effectiveness of treatment of HCV or any of the other indications discussed above. siNA molecules targeting RNAs associated with

HCV infection can be used individually or in combination with other therapies such as interferons and polyethylene glycol interferons and to achieve enhanced efficacy.

Example 13: Diagnostic uses

The siNA molecules of the invention can be used in a variety of diagnostic applications, such as in the identification of molecular targets (e.g., RNA) in a variety of applications, for example, in clinical, industrial, environmental, agricultural and/or research settings. Such diagnostic use of siNA molecules involves utilizing reconstituted RNAi systems, for example, using cellular lysates or partially purified cellular lysates. siNA molecules of this invention can be used as diagnostic tools to examine genetic drift and mutations within diseased cells or to detect the presence of endogenous or exogenous, for example viral, RNA in a cell. The close relationship between siNA activity and the structure of the target RNA allows the detection of mutations in any region of the molecule, which alters the base-pairing and three-dimensional structure of the target RNA. By using multiple siNA molecules described in this invention, one can map nucleotide changes, which are important to RNA structure and function *in vitro*, as well as in cells and tissues. Cleavage of target RNAs with siNA molecules can be used to inhibit gene expression and define the role of specified gene products in the progression of disease or infection. In this manner, other genetic targets can be defined as important mediators of the disease. These experiments will lead to better treatment of the disease progression by affording the possibility of combination therapies (e.g., multiple siNA molecules targeted to different genes, siNA molecules coupled with known small molecule inhibitors, or intermittent treatment with combinations siNA molecules and/or other chemical or biological molecules). Other *in vitro* uses of siNA molecules of this invention are well known in the art, and include detection of the presence of mRNAs associated with a disease, infection, or related condition. Such RNA is detected by determining the presence of a cleavage product after treatment with a siNA using standard methodologies, for example, fluorescence resonance emission transfer (FRET).

In a specific example, siNA molecules that cleave only wild-type or mutant forms of the target RNA are used for the assay. The first siNA molecules (*i.e.*, those that cleave only wild-type forms of target RNA) are used to identify wild-type RNA present

in the sample and the second siNA molecules (*i.e.*, those that cleave only mutant forms of target RNA) are used to identify mutant RNA in the sample. As reaction controls, synthetic substrates of both wild-type and mutant RNA are cleaved by both siNA molecules to demonstrate the relative siNA efficiencies in the reactions and the absence
5 of cleavage of the "non-targeted" RNA species. The cleavage products from the synthetic substrates also serve to generate size markers for the analysis of wild-type and mutant RNAs in the sample population. Thus, each analysis requires two siNA molecules, two substrates and one unknown sample, which is combined into six reactions. The presence of cleavage products is determined using an RNase protection
10 assay so that full-length and cleavage fragments of each RNA can be analyzed in one lane of a polyacrylamide gel. It is not absolutely required to quantify the results to gain insight into the expression of mutant RNAs and putative risk of the desired phenotypic changes in target cells. The expression of mRNA whose protein product is implicated in the development of the phenotype (*i.e.*, disease related or infection related) is adequate to
15 establish risk. If probes of comparable specific activity are used for both transcripts, then a qualitative comparison of RNA levels is adequate and decreases the cost of the initial diagnosis. Higher mutant form to wild-type ratios are correlated with higher risk whether RNA levels are compared qualitatively or quantitatively.

All patents and publications mentioned in the specification are indicative of the
20 levels of skill of those skilled in the art to which the invention pertains. All references cited in this disclosure are incorporated by reference to the same extent as if each reference had been incorporated by reference in its entirety individually.

One skilled in the art would readily appreciate that the present invention is well adapted to carry out the objects and obtain the ends and advantages mentioned, as well as
25 those inherent therein. The methods and compositions described herein as presently representative of preferred embodiments are exemplary and are not intended as limitations on the scope of the invention. Changes therein and other uses will occur to those skilled in the art, which are encompassed within the spirit of the invention, are defined by the scope of the claims.

30 It will be readily apparent to one skilled in the art that varying substitutions and modifications can be made to the invention disclosed herein without departing from the

scope and spirit of the invention. Thus, such additional embodiments are within the scope of the present invention and the following claims. The present invention teaches one skilled in the art to test various combinations and/or substitutions of chemical modifications described herein toward generating nucleic acid constructs with improved activity for mediating RNAi activity. Such improved activity can comprise improved stability, improved bioavailability, and/or improved activation of cellular responses mediating RNAi. Therefore, the specific embodiments described herein are not limiting and one skilled in the art can readily appreciate that specific combinations of the modifications described herein can be tested without undue experimentation toward identifying siNA molecules with improved RNAi activity.

The invention illustratively described herein suitably can be practiced in the absence of any element or elements, limitation or limitations that are not specifically disclosed herein. Thus, for example, in each instance herein any of the terms "comprising", "consisting essentially of", and "consisting of" may be replaced with either of the other two terms. The terms and expressions which have been employed are used as terms of description and not of limitation, and there is no intention that in the use of such terms and expressions of excluding any equivalents of the features shown and described or portions thereof, but it is recognized that various modifications are possible within the scope of the invention claimed. Thus, it should be understood that although the present invention has been specifically disclosed by preferred embodiments, optional features, modification and variation of the concepts herein disclosed may be resorted to by those skilled in the art, and that such modifications and variations are considered to be within the scope of this invention as defined by the description and the appended claims.

In addition, where features or aspects of the invention are described in terms of Markush groups or other grouping of alternatives, those skilled in the art will recognize that the invention is also thereby described in terms of any individual member or subgroup of members of the Markush group or other group.

Table I: HCV Accession Numbers

Seq Name	Acc#	LOCUS
gi 329763 gb M84754.1 HPCGENANTI	M84754.1	HPCGENANTI
gi 567059 gb U16362.1 HCU16362	U16362.1	HCU16362
gi 5918956 gb AF165059.1 AF165059	AF165059.1	AF165059
gi 385583 gb S62220.1 S62220	S62220.1	S62220
gi 6010587 gb AF177040.1 AF177040	AF177040.1	AF177040
gi 5748510 emb AJ238800.1 HCJ238800	AJ238800.1	HCJ238800
gi 7650221 gb AF207752.1 AF207752	AF207752.1	AF207752
gi 11559454 dbj AB049094.1 AB049094	AB049094.1	AB049094
gi 3550760 dbj D84263.1 D84263	D84263.1	D84263
gi 221610 dbj D90208.1 HPCJCG	D90208.1	HPCJCG
gi 558520 dbj D28917.1 HPCK3A	D28917.1	HPCK3A
gi 2176577 dbj E08461.1 E08461	E08461.1	E08461
gi 6707285 gb AF169005.1 AF169005	AF169005.1	AF169005
gi 12309923 emb AX057094.1 AX057094	AX057094.1	AX057094
gi 6010585 gb AF177039.1 AF177039	AF177039.1	AF177039
gi 7329202 gb AF238482.1 AF238482	AF238482.1	AF238482
gi 11559464 dbj AB049099.1 AB049099	AB049099.1	AB049099
gi 5918932 gb AF165047.1 AF165047	AF165047.1	AF165047
gi 5918946 gb AF165054.1 AF165054	AF165054.1	AF165054
gi 7650233 gb AF207758.1 AF207758	AF207758.1	AF207758
gi 19568932 gb AF483269.1	AF483269.1	
gi 7650247 gb AF207765.1 AF207765	AF207765.1	AF207765
gi 12309919 emb AX057086.1 AX057086	AX057086.1	AX057086
gi 5708597 dbj E10839.1 E10839	E10839.1	E10839
gi 2327074 gb AF011753.1 AF011753	AF011753.1	AF011753
gi 12310062 emb AX057317.1 AX057317	AX057317.1	AX057317
gi 221606 dbj D10750.1 HPCJ491	D10750.1	HPCJ491
gi 2174448 dbj E06261.1 E06261	E06261.1	E06261
gi 3098640 gb AF054251.1 AF054251	AF054251.1	AF054251
gi 18027684 gb AF313916.1 AF313916	AF313916.1	AF313916
gi 329873 gb M62321.1 HPCPLYPRE	M62321.1	HPCPLYPRE
gi 464177 dbj D14853.1 HPCCGS	D14853.1	HPCCGS
gi 15422182 gb AY051292.1	AY051292.1	
gi 676877 dbj D49374.1 HPCFG	D49374.1	HPCFG
gi 1030706 dbj D50480.1 HPCK1R1	D50480.1	HPCK1R1
gi 7650223 gb AF207753.1 AF207753	AF207753.1	AF207753
gi 7650237 gb AF207760.1 AF207760	AF207760.1	AF207760
gi 11559444 dbj AB049089.1 AB049089	AB049089.1	AB049089
gi 3550762 dbj D84264.1 D84264	D84264.1	D84264
gi 12831192 gb AF333324.1 AF333324	AF333324.1	AF333324
gi 13122265 dbj AB047641.1 AB047641	AB047641.1	AB047641
gi 7329204 gb AF238483.1 AF238483	AF238483.1	AF238483
gi 11559468 dbj AB049101.1 AB049101	AB049101.1	AB049101
gi 5918934 gb AF165048.1 AF165048	AF165048.1	AF165048
gi 5918948 gb AF165055.1 AF165055	AF165055.1	AF165055
gi 7650235 gb AF207759.1 AF207759	AF207759.1	AF207759
gi 7650249 gb AF207766.1 AF207766	AF207766.1	AF207766
gi 9843676 emb AJ278830.1 HEC278830	AJ278830.1	HEC278830
gi 11559450 dbj AB049092.1 AB049092	AB049092.1	AB049092
gi 2943783 dbj D89815.1 D89815	D89815.1	D89815

gi 9626438 ref NC_001433.1	NC_001433.1	
gi 12310134 emb AX057395.1 AX057395	AX057395.1	AX057395
gi 11559460 db AB049097.1 AB049097	AB049097.1	AB049097
gi 12309922 emb AX057092.1 AX057092	AX057092.1	AX057092
gi 2174644 db E06457.1 E06457	E06457.1	E06457
gi 2176559 db E08443.1 E08443	E08443.1	E08443
gi 5918960 gb AF165061.1 AF165061	AF165061.1	AF165061
gi 2326454 emb Y12083.1 HCV12083	Y12083.1	HCV12083
gi 5918938 gb AF165050.1 AF165050	AF165050.1	AF165050
gi 7650225 gb AF207754.1 AF207754	AF207754.1	AF207754
gi 7650261 gb AF207772.1 AF207772	AF207772.1	AF207772
gi 1030704 db D50485.1 HPCK1S2	D50485.1	HPCK1S2
gi 3550758 db D84262.1 D84262	D84262.1	D84262
gi 7650239 gb AF207761.1 AF207761	AF207761.1	AF207761
gi 3550764 db D84265.1 D84265	D84265.1	D84265
gi 7329206 gb AF238484.1 AF238484	AF238484.1	AF238484
gi 2176516 db E08399.1 E08399	E08399.1	E08399
gi 5918936 gb AF165049.1 AF165049	AF165049.1	AF165049
gi 11559446 db AB049090.1 AB049090	AB049090.1	AB049090
gi 5441837 emb AJ242653.1 SSE242653	AJ242653.1	SSE242653
gi 3098641 gb AF054252.1 AF054252	AF054252.1	AF054252
gi 4753720 emb AJ132997.1 HCV132997	AJ132997.1	HCV132997
gi 5420376 emb AJ238799.1 HCJ238799	AJ238799.1	HCJ238799
gi 11559440 db AB049087.1 AB049087	AB049087.1	AB049087
gi 15529110 gb AY045702.1	AY045702.1	
gi 560788 db D30613.1 HPCPP	D30613.1	HPCPP
gi 11225869 emb AX036253.1 AX036253	AX036253.1	AX036253
gi 11559456 db AB049095.1 AB049095	AB049095.1	AB049095
gi 329770 gb M58335.1 HPCHUMR	M58335.1	HPCHUMR
gi 6707279 gb AF169002.1 AF169002	AF169002.1	AF169002
gi 221586 db D10749.1 HPCHCJ1	D10749.1	HPCHCJ1
gi 2171981 db E03766.1 E03766	E03766.1	E03766
gi 6010579 gb AF177036.1 AF177036	AF177036.1	AF177036
gi 1030703 db D50484.1 HPCK1S3	D50484.1	HPCK1S3
gi 3098650 gb AF054257.1 AF054257	AF054257.1	AF054257
gi 5821154 db AB016785.1 AB016785	AB016785.1	AB016785
gi 5918962 gb AF165062.1 AF165062	AF165062.1	AF165062
gi 7650227 gb AF207755.1 AF207755	AF207755.1	AF207755
gi 7650263 gb AF207773.1 AF207773	AF207773.1	AF207773
gi 1183030 db D63822.1 HPCJK046E2	D63822.1	HPCJK046E2
gi 13122271 db AB047644.1 AB047644	AB047644.1	AB047644
gi 2443428 gb U89019.1 HCU89019	U89019.1	HCU89019
gi 2462303 emb Y13184.1 HCV1480	Y13184.1	HCV1480
gi 7329208 gb AF238485.1 AF238485	AF238485.1	AF238485
gi 1160327 db D14484.1 HPCJRNA	D14484.1	HPCJRNA
gi 12309921 emb AX057090.1 AX057090	AX057090.1	AX057090
gi 3098643 gb AF054253.1 AF054253	AF054253.1	AF054253
gi 21397075 gb AF511948.1	AF511948.1	
gi 1030701 db D50482.1 HPCK1R3	D50482.1	HPCK1R3
gi 1030702 db D50483.1 HPCK1S1	D50483.1	HPCK1S1
gi 3098632 gb AF054247.1 AF054247	AF054247.1	AF054247
gi 59478 emb X61596.1 HCVJK1G	X61596.1	HCVJK1G
gi 3098652 gb AF054258.1 AF054258	AF054258.1	AF054258
gi 5918950 gb AF165056.1 AF165056	AF165056.1	AF165056
gi 7650251 gb AF207767.1 AF207767	AF207767.1	AF207767

gi 5918964 gb AF165063.1 AF165063	AF165063.1	AF165063
gi 5918928 gb AF165045.1 AF165045	AF165045.1	AF165045
gi 5532421 gb AF139594.1 AF139594	AF139594.1	AF139594
gi 13122267 db AB047642.1 AB047642	AB047642.1	AB047642
gi 5441831 emb AJ242651.1 SSE242651	AJ242651.1	SSE242651
gi 7650265 gb AF207774.1 AF207774	AF207774.1	AF207774
gi 7650229 gb AF207756.1 AF207756	AF207756.1	AF207756
gi 1183032 db D63821.1 HPCJK049E1	D63821.1	HPCJK049E1
gi 2175714 db E07579.1 E07579	E07579.1	E07579
gi 1212741 db D45172.1 HPCHCPO	D45172.1	HPCHCPO
gi 5708511 db E05027.1 E05027	E05027.1	E05027
gi 1483141 db D50409.1 D50409	D50409.1	D50409
gi 13122261 db AB047639.1 AB047639	AB047639.1	AB047639
gi 6521008 db AB031663.1 AB031663	AB031663.1	AB031663
gi 633201 emb X76918.1 HCV CENS1	X76918.1	HCV CENS1
gi 329737 gb M67463.1 HPCCGAA	M67463.1	HPCCGAA
gi 11559452 db AB049093.1 AB049093	AB049093.1	AB049093
gi 13619567 emb AX100563.1 AX100563	AX100563.1	AX100563
gi 221604 db D13558.1 HPCJ483	D13558.1	HPCJ483
gi 11225872 emb AX036256.1 AX036256	AX036256.1	AX036256
gi 1749761 db D89872.1 D89872	D89872.1	D89872
gi 5918940 gb AF165051.1 AF165051	AF165051.1	AF165051
gi 4753718 emb AJ132996.1 HCV132996	AJ132996.1	HCV132996
gi 7650241 gb AF207762.1 AF207762	AF207762.1	AF207762
gi 3098645 gb AF054254.1 AF054254	AF054254.1	AF054254
gi 9930556 gb AF290978.1 AF290978	AF290978.1	AF290978
gi 11559462 db AB049098.1 AB049098	AB049098.1	AB049098
gi 2764397 emb AJ000009.1 HCV POLYP	AJ000009.1	HCV POLYP
gi 221608 db D10988.1 HPCJ8G	D10988.1	HPCJ8G
gi 3098634 gb AF054248.1 AF054248	AF054248.1	AF054248
gi 221650 db D00944.1 HPCPOLP	D00944.1	HPCPOLP
gi 306286 gb M96362.1 HPCUNKCDS	M96362.1	HPCUNKCDS
gi 3098654 gb AF054259.1 AF054259	AF054259.1	AF054259
gi 5918952 gb AF165057.1 AF165057	AF165057.1	AF165057
gi 7650253 gb AF207768.1 AF207768	AF207768.1	AF207768
gi 5918966 gb AF165064.1 AF165064	AF165064.1	AF165064
gi 15487693 gb AF356827.1 AF356827	AF356827.1	AF356827
gi 5738246 gb AF176573.1 AF176573	AF176573.1	AF176573
gi 11559448 db AB049091.1 AB049091	AB049091.1	AB049091
gi 21397077 gb AF511950.1	AF511950.1	
gi 3098638 gb AF054250.1 AF054250	AF054250.1	AF054250
gi 6707281 gb AF169003.1 AF169003	AF169003.1	AF169003
gi 329739 gb L02836.1 HPCCGENOM	L02836.1	HPCCGENOM
gi 6010581 gb AF177037.1 AF177037	AF177037.1	AF177037
gi 11559442 db AB049088.1 AB049088	AB049088.1	AB049088
gi 21397076 gb AF511949.1	AF511949.1	
gi 1030705 db D50481.1 HPCK1R2	D50481.1	HPCK1R2
gi 2176384 db E08264.1 E08264	E08264.1	E08264
gi 3660725 gb AF064490.1 AF064490	AF064490.1	AF064490
gi 2252489 emb Y11604.1 HCV4APOLY	Y11604.1	HCV4APOLY
gi 5918942 gb AF165052.1 AF165052	AF165052.1	AF165052
gi 2895898 gb AF046866.1 AF046866	AF046866.1	AF046866
gi 7650243 gb AF207763.1 AF207763	AF207763.1	AF207763
gi 11559458 db AB049096.1 AB049096	AB049096.1	AB049096
gi 13122263 db AB047640.1 AB047640	AB047640.1	AB047640

gi 5708574 dbj E08263.1 E08263	E08263.1	E08263
gi 7650257 gb AF207770.1 AF207770	AF207770.1	AF207770
gi 3098647 gb AF054255.1 AF054255	AF054255.1	AF054255
gi 11559466 dbj AB049100.1 AB049100	AB049100.1	AB049100
gi 1181831 gb U45476.1 HCU45476	U45476.1	HCU45476
gi 2327070 gb AF011751.1 AF011751	AF011751.1	AF011751
gi 3098636 gb AF054249.1 AF054249	AF054249.1	AF054249
gi 7329210 gb AF238486.1 AF238486	AF238486.1	AF238486
gi 221612 dbj D11168.1 HPCJTA	D11168.1	HPCJTA
gi 960359 dbj D63857.1 HPVHCVN	D63857.1	HPVHCVN
gi 13122273 dbj AB047645.1 AB047645	AB047645.1	AB047645
gi 5918954 gb AF165058.1 AF165058	AF165058.1	AF165058
gi 7650255 gb AF207769.1 AF207769	AF207769.1	AF207769
gi 437107 gb U01214.1 HCU01214	U01214.1	HCU01214
gi 471116 dbj D10934.1 HPCRNA	D10934.1	HPCRNA
gi 13026028 dbj E66593.1 E66593	E66593.1	E66593
gi 2316097 gb AF009606.1 AF009606	AF009606.1	AF009606
gi 6707283 gb AF169004.1 AF169004	AF169004.1	AF169004
gi 514395 dbj D17763.1 HPCEGS	D17763.1	HPCEGS
gi 9757541 dbj AB030907.1 AB030907	AB030907.1	AB030907
gi 7329200 gb AF238481.1 AF238481	AF238481.1	AF238481
gi 6010583 gb AF177038.1 AF177038	AF177038.1	AF177038
gi 2172621 dbj E04420.1 E04420	E04420.1	E04420
gi 8926244 gb AF271632.1 AF271632	AF271632.1	AF271632
gi 5918930 gb AF165046.1 AF165046	AF165046.1	AF165046
gi 7650231 gb AF207757.1 AF207757	AF207757.1	AF207757
gi 5918944 gb AF165053.1 AF165053	AF165053.1	AF165053
gi 7650245 gb AF207764.1 AF207764	AF207764.1	AF207764
gi 12309920 emb AX057088.1 AX057088	AX057088.1	AX057088
gi 5918958 gb AF165060.1 AF165060	AF165060.1	AF165060
gi 7650259 gb AF207771.1 AF207771	AF207771.1	AF207771
gi 7341102 gb AF208024.1 AF208024	AF208024.1	AF208024
gi 3098649 gb AF054256.1 AF054256	AF054256.1	AF054256
gi 1944375 dbj D85516.1 D85516	D85516.1	D85516
gi 2327072 gb AF011752.1 AF011752	AF011752.1	AF011752
gi 221614 dbj D11355.1 HPCJTB	D11355.1	HPCJTB
gi 13122269 dbj AB047643.1 AB047643	AB047643.1	AB047643

Table II: HCV siNA and Target Sequences

NM_000594 (hHCV)					
Sequence	SeqID	Upper seq	Seq ID	Lower seq	Seq ID
GCCCCGGGAGGUCUCGUAG	1	GCCCCGGGAGGUCUCGUAG	1	CUACGAGACCUCGCGGGC	697
UGUGGUACUGCCUGAUAGG	2	UGUGGUACUGCCUGAUAGG	2	CCUAUCAGGCAGUACCACA	698
UUUGGUACUGCCUGAUAG	3	UUUGGUACUGCCUGAUAG	3	CUAUCAGGCAGUACCACAA	699
CCCCGGGAGGUCUCGUAGA	4	CCCCGGGAGGUCUCGUAGA	4	UCUACGAGACCUCGCGGG	700
GUGGUACUGCCUGAUAGG	5	GUGGUACUGCCUGAUAGG	5	CCCUAUCAGGCAGUACCAC	701
CUGCCUGAUAGGUGCUUG	6	CUGCCUGAUAGGUGCUUG	6	CAAGCACCCUAUCAGGCAG	702
CCUUGUGGUACUGCCUGAU	7	CCUUGUGGUACUGCCUGAU	7	AUCAGGCAGUACCACAAGG	703
GCGAAAGGCCUUGUGGUAC	8	GCGAAAGGCCUUGUGGUAC	8	GUACCACAAGGCCUUCGCG	704
UACUGCCUGAUAGGUGCU	9	UACUGCCUGAUAGGUGCU	9	AGCACCCUAUCAGGCAGUA	705
GGUACUGCCUGAUAGGUG	10	GGUACUGCCUGAUAGGUG	10	CACCCUAUCAGGCAGUACC	706
AAAGGCCUUGUGGUACUGC	11	AAAGGCCUUGUGGUACUGC	11	GCAGUACCACAAGGCCUUU	707
AAGGCCUUGUGGUACUGCC	12	AAGGCCUUGUGGUACUGCC	12	GGCAGUACCACAAGGCCUU	708
CUUGUGGUACUGCCUGAU	13	CUUGUGGUACUGCCUGAU	13	UAUCAGGCAGUACCACAAG	709
AGGCCUUGUGGUACUGCCU	14	AGGCCUUGUGGUACUGCCU	14	AGGCAGUACCACAAGGCCU	710
GUACUGCCUGAUAGGUGC	15	GUACUGCCUGAUAGGUGC	15	GCACCCUAUCAGGCAGUAC	711
ACUGCCUGAUAGGUGCUU	16	ACUGCCUGAUAGGUGCUU	16	AAGCACCCUAUCAGGCAGU	712
CUUGCGAGUGCCCCGGGAG	17	CUUGCGAGUGCCCCGGGAG	17	CUCCCGGGGCACUCGCAAG	713
CUGAUAGGUGCUUCCGAG	18	CUGAUAGGUGCUUCCGAG	18	CUCGCAAGCACCCUAUCAG	714
UUGCGAGUGCCCCGGGAGG	19	UUGCGAGUGCCCCGGGAGG	19	CCUCCCGGGGCACUCGCAA	715
CCUGAUAGGUGCUUUGCGA	20	CCUGAUAGGUGCUUUGCGA	20	UCGCAAGCACCCUAUCAGG	716
GGCCUUGUGGUACUGCCUG	21	GGCCUUGUGGUACUGCCUG	21	CAGGCAGUACCACAAGGCC	717
GCUUGCGAGUGCCCCGGGA	22	GCUUGCGAGUGCCCCGGGA	22	UCCCGGGGCACUCGCAAGC	718
UGCCUGAUAGGUGCUUUGC	23	UGCCUGAUAGGUGCUUUGC	23	GCAAGCACCCUAUCAGGCA	719
GAAAGGCCUUGUGGUACUG	24	GAAAGGCCUUGUGGUACUG	24	CAGUACCACAAGGCCUUUC	720
GCCUGAUAGGUGCUUUGCG	25	GCCUGAUAGGUGCUUUGCG	25	CGCAAGCACCCUAUCAGGC	721
CGAAAGGCCUUGUGGUACU	26	CGAAAGGCCUUGUGGUACU	26	AGUACCACAAGGCCUUUCG	722
GCCUUGUGGUACUGCCUGA	27	GCCUUGUGGUACUGCCUGA	27	UCAGGCAGUACCACAAGGC	723
GAGUGCCCCGGGAGGUCUC	28	GAGUGCCCCGGGAGGUCUC	28	GAGACCUCCCGGGGCACUC	724
CCCGGAGGUCUCGUAGAC	29	CCCGGAGGUCUCGUAGAC	29	GUCUACGAGACCUCGCGGG	725
UGCGAGUGCCCCGGGAGGU	30	UGCGAGUGCCCCGGGAGGU	30	ACCUCCCGGGGCACUCGCA	726

UGGUACUGCCUGAUAGGGU	31	UGGUACUGCCUGAUAGGGU	31	ACCUAUCAGGCAGUACCA	727
CCGGUGAGUACACCGGAU	32	CCGGUGAGUACACCGGAU	32	AUUCGGUGUACUCACCGG	728
GCGAGUGCCCGGAGGUC	33	GCGAGUGCCCGGAGGUC	33	GACCUCCCGGGGCACUCGC	729
CGAGUGCCCGGAGGUCU	34	CGAGUGCCCGGAGGUCU	34	AGACUCCCGGGGCACUCG	730
UGCCCGGGAGGUCUCGUA	35	UGCCCGGGAGGUCUCGUA	35	UACGAGACCUCCCGGGCA	731
GUGCCCGGGAGGUCUCGU	36	GUGCCCGGGAGGUCUCGU	36	ACGAGACCUCCCGGGGCAC	732
AGUGCCCGGGAGGUCUCG	37	AGUGCCCGGGAGGUCUCG	37	CGAGACCUCCCGGGGCACU	733
CCGGAGGUCUCGUAGACC	38	CCGGAGGUCUCGUAGACC	38	GGUCUACGAGACCUCCCGG	734
UGAUAGGGUGCUUGCGAGU	39	UGAUAGGGUGCUUGCGAGU	39	ACUCGCAAGCACCCUAUA	735
GUGCUUGCGAGUGCCCCGG	40	GUGCUUGCGAGUGCCCCGG	40	CCGGGCACUCGCAAGCAC	736
AUAGGGUGCUUGCGAGUGC	41	AUAGGGUGCUUGCGAGUGC	41	GCACUCGCAAGCACCCUAU	737
GGGUGCUUGCGAGUGCCCC	42	GGGUGCUUGCGAGUGCCCC	42	GGGGCACUCGCAAGCACCC	738
CGGAGGUCUCGUAGACCG	43	CGGAGGUCUCGUAGACCG	43	CGGUCUACGAGACCUCCCG	739
GGGAGGUCUCGUAGACCGU	44	GGGAGGUCUCGUAGACCGU	44	ACGGUCUACGAGACCUCCC	740
GAUAGGGUGCUUGCGAGUG	45	GAUAGGGUGCUUGCGAGUG	45	CACUCGCAAGCACCCUAUC	741
GGAGGUCUCGUAGACCGUG	46	GGAGGUCUCGUAGACCGUG	46	CACGGUCUACGAGACCUCC	742
AGGGUGCUUGCGAGUGCCC	47	AGGGUGCUUGCGAGUGCCC	47	GGGCACUCGCAAGCACCCU	743
UGCUGCGAGUGCCCCGGG	48	UGCUGCGAGUGCCCCGGG	48	CCCGGGCACUCGCAAGCA	744
GGUGCUUGCGAGUGCCCCG	49	GGUGCUUGCGAGUGCCCCG	49	CGGGGCACUCGCAAGCACCC	745
UAGGGUGCUUGCGAGUGCC	50	UAGGGUGCUUGCGAGUGCC	50	GGCACUCGCAAGCACCCUA	746
AGGUCUCGUAGACCGUGCA	51	AGGUCUCGUAGACCGUGCA	51	UGCACGGUCUACGAGACCU	747
GAGGUCUCGUAGACCGUGC	52	GAGGUCUCGUAGACCGUGC	52	GCACGGUCUACGAGACCU	748
GGAACCGGUGAGUACACCG	53	GGAACCGGUGAGUACACCG	53	CGGUGUACUACCGGUUCC	749
CGGAACCGGUGAGUACACC	54	CGGAACCGGUGAGUACACC	54	GGUGUACUACCGGUUCCG	750
CGGUGAGUACACCGGAUU	55	CGGUGAGUACACCGGAUU	55	AAUUCGGUGUACUCACCGG	751
GCGGAACCGGUGAGUACAC	56	GCGGAACCGGUGAGUACAC	56	GUGUACUACCGGUUCCCG	752
AACCGGUGAGUACACCGGA	57	AACCGGUGAGUACACCGGA	57	UCCGGUGUACUCACCGGUU	753
ACCGGUGAGUACACCGGAA	58	ACCGGUGAGUACACCGGAA	58	UUCCGGUGUACUCACCGGU	754
CUGCGGAACCGGUGAGUAC	59	CUGCGGAACCGGUGAGUAC	59	GUACUACCGGUUCCCGCAG	755
GUCUGCGGAACCGGUGAGU	60	GUCUGCGGAACCGGUGAGU	60	ACUCACCGGUUCCCGCAGAC	756
GAACCGGUGAGUACACCGG	61	GAACCGGUGAGUACACCGG	61	CCGGUGUACUCACCGGUUC	757
UGCGGAACCGGUGAGUACA	62	UGCGGAACCGGUGAGUACA	62	UGUACUCACCGGUUCCGCA	758
UCUGCGGAACCGGUGAGUA	63	UCUGCGGAACCGGUGAGUA	63	UACUCACCGGUUCCCGCAGA	759
GGGAGAGCCAUAGUGGUCU	64	GGGAGAGCCAUAGUGGUCU	64	AGACCACUAGGUCUCUCCC	760
GUGGUCUGCGGAACCGGUG	65	GUGGUCUGCGGAACCGGUG	65	CACCGGUUCCCGCAGACCAC	761
GGUCUGCGGAACCGGUGAG	66	GGUCUGCGGAACCGGUGAG	66	CUCACCGGUUCCCGCAGACC	762

CGGGAGCCAUAGUGGUC	67	CGGGAGAGCCAUAGUGGUC	67	GACCACUAGGCUCUCCCG	763
CCGGAGAGCCAUAGUGGU	68	CCGGAGAGCCAUAGUGGU	68	ACCACUAGGCUCUCCCGG	764
UGGUCUGCGGAACCGGUGA	69	UGGUCUGCGGAACCGGUGA	69	UCACCGGUUCCGCAGACCA	765
GUGAGUACACCGGAUUGC	70	GUGAGUACACCGGAUUGC	70	GCAAUUCCGGUGUACUCAC	766
UGAGUACACCGGAUUGCC	71	UGAGUACACCGGAUUGCC	71	GGCAUUUCCGGUGUACUCA	767
GGUGAGUACACCGGAUUG	72	GGUGAGUACACCGGAUUG	72	CAAUUCCGGUGUACUCACC	768
GAGCCAUAGUGGUCUGCGG	73	GAGCCAUAGUGGUCUGCGG	73	CCGCAGACCACUAGGCUC	769
AGAGCCAUAGUGGUCUGCG	74	AGAGCCAUAGUGGUCUGCG	74	CGCAGACCACUAGGCUCU	770
UAGUGGUCUGCGGAACCGG	75	UAGUGGUCUGCGGAACCGG	75	CCGUUCCGCAGACCACUA	771
AUAGUGGUCUGCGGAACCG	76	AUAGUGGUCUGCGGAACCG	76	CGGUUCCGCAGACCACUAU	772
GAGAGCCAUAGUGGUCUGC	77	GAGAGCCAUAGUGGUCUGC	77	GCAGACCACUAGGCUCUC	773
GCCAUAGUGGUCUGCGGAA	78	GCCAUAGUGGUCUGCGGAA	78	UUCGCAGACCACUAGGC	774
AGUGGUCUGCGGAACCGGU	79	AGUGGUCUGCGGAACCGGU	79	ACCGGUUCCGCAGACCACU	775
CAUAGUGGUCUGCGGAACC	80	CAUAGUGGUCUGCGGAACC	80	GGUCCGCAGACCACUAG	776
AGCCAUAGUGGUCUGCGGA	81	AGCCAUAGUGGUCUGCGGA	81	UCCGCAGACCACUAGGCU	777
CCAUAGUGGUCUGCGGAAC	82	CCAUAGUGGUCUGCGGAAC	82	GUUCCGCAGACCACUAGG	778
CCCCUCCCGGAGAGCCAU	83	CCCCUCCCGGAGAGCCAU	83	AUGGUCUCCCGGAGGGG	779
GGAGAGCCAUAGUGGUCUG	84	GGAGAGCCAUAGUGGUCUG	84	CAGACCACUAGGCUCUC	780
CCCGGAGAGCCAUAGUGG	85	CCCGGAGAGCCAUAGUGG	85	CCACUAGGCUCUCCCGGG	781
CCCCUCCCGGAGAGCCA	86	CCCCUCCCGGAGAGCCA	86	UGGUCUCCCGGAGGGG	782
UCCCGGAGAGCCAUAGUG	87	UCCCGGAGAGCCAUAGUG	87	CACUAGGCUCUCCCGGGA	783
CCCCCUCCCGGAGAGCC	88	CCCCCUCCCGGAGAGCC	88	GGCUCUCCCGGAGGGGG	784
CCCUCCCGGAGAGCCAU	89	CCCUCCCGGAGAGCCAU	89	UAUGGCUCUCCCGGAGGG	785
CCUCCCGGAGAGCCAUAG	90	CCUCCCGGAGAGCCAUAG	90	CUAUGGCUCUCCCGGAGG	786
CUCCCGGAGAGCCAUAGU	91	CUCCCGGAGAGCCAUAGU	91	ACUAUGGCUCUCCCGGAG	787
UGUUGCCGCGAGGGCCC	92	UGUUGCCGCGAGGGCCC	92	GGCCCCUGCGCGGCAACA	788
CCCCCCCUCGCGGAGAGC	93	CCCCCCCUCGCGGAGAGC	93	GCUCUCCCGGAGGGGGG	789
CAUGGCGUUAUAGUAGUG	94	CAUGGCGUUAUAGUAGUG	94	CACUCAUACUAAACGCCAUG	790
UAGCCAUUGGCGUUAUAG	95	UAGCCAUUGGCGUUAUAG	95	CAUACUAAACGCCAUGGCUA	791
AGCCAUUGGCGUUAUAGA	96	AGCCAUUGGCGUUAUAGA	96	UCAUACUAAACGCCAUGGCU	792
CCAUGGCGUUAUAGUAGU	97	CCAUGGCGUUAUAGUAGU	97	ACUCAUACUAAACGCCAUGG	793
AUGGCGUUAUAGUAGUGU	98	AUGGCGUUAUAGUAGUGU	98	ACACUCAUACUAAACGCCAU	794
AAGCGUCUAGCCAUUGCGU	99	AAGCGUCUAGCCAUUGCGU	99	ACGCCAUGGCUAGACGCUU	795
GUCUAGCCAUUGGCGUUAU	100	GUCUAGCCAUUGGCGUUAU	100	ACUAAACGCCAUGGCUAGAC	796
AAAGCGUCUAGCCAUUGCG	101	AAAGCGUCUAGCCAUUGCG	101	CGCCAUGGCUAGACGCUUU	797
GCGUCUAGCCAUUGGCGUUA	102	GCGUCUAGCCAUUGGCGUUA	102	UAACGCCAUGGCUAGACGC	798

GCCAUGGCGUUAGUAG	103	GCCAUGGCGUUAGUAG	103	CUCAUACUAAAGCCAUAGC	799
AGCGUUAAGCCAUAGCGUU	104	AGCGUUAAGCCAUAGCGUU	104	AAGCCAUAGCGUAGACGCU	800
CGUCUAGCCAUAGCGUUAG	105	CGUCUAGCCAUAGCGUUAG	105	CUAACGCCAUAGCGUAGACG	801
UCUAGCCAUAGCGUUAGUA	106	UCUAGCCAUAGCGUUAGUA	106	UACUAAAGCCAUAGCGUAGA	802
GAAAGCGUUAAGCCAUAGC	107	GAAAGCGUUAAGCCAUAGC	107	GCCAUAGCGUAGACGCUUUC	803
CUAGCCAUAGCGUUAGUAU	108	CUAGCCAUAGCGUUAGUAU	108	AUACUAAAGCCAUAGCGUAG	804
CACUCCCCUGUAGGAACU	109	CACUCCCCUGUAGGAACU	109	AGUCCUCACAGGGGAGUG	805
ACCUCAAAGAAACCAAA	110	ACCUCAAAGAAACCAAA	110	UUUGUUUUUUUUUGAGGU	806
CGCAGAAAGCGUUAAGCCA	111	CGCAGAAAGCGUUAAGCCA	111	UGGCUAGACGCUUUCUGCG	807
GGGUAAGGUCAUCGAUACC	112	GGGUAAGGUCAUCGAUACC	112	GGUAUCGAUGACCUUACCC	808
CAGAAAGCGUUAAGCCAU	113	CAGAAAGCGUUAAGCCAU	113	CAUGGCUAGACGCUUUCUG	809
AAACCUCAAAGAAACCCA	114	AAACCUCAAAGAAACCCA	114	UGGUUUUUUUUUUGAGGUU	810
GCAGAAAGCGUUAAGCCAU	115	GCAGAAAGCGUUAAGCCAU	115	AUGGCUAGACGCUUUCUGC	811
AGAAAGCGUUAAGCCAU	116	AGAAAGCGUUAAGCCAU	116	CCAUGGCUAGACGCUUUCU	812
ACGCAGAAAGCGUUAAGCC	117	ACGCAGAAAGCGUUAAGCC	117	GGCUAGACGCUUUCUGCGU	813
AACCUCAAAGAAACCAAA	118	AACCUCAAAGAAACCAAA	118	UUGUUUUUUUUUUUGAGGUU	814
UGGUUAAGGUCAUCGAUAC	119	UGGUUAAGGUCAUCGAUAC	119	GUUUCGAUGACCUUACCCA	815
GUUAGGUCAUCGAUACCCU	120	GUUAGGUCAUCGAUACCCU	120	AGGUUAUCGAUGACCUUAC	816
UUCACGCAGAAAGCGUUA	121	UUCACGCAGAAAGCGUUA	121	UAGACGCUUUCUGCGUGAA	817
GGUUAAGGUCAUCGAUACCC	122	GGUUAAGGUCAUCGAUACCC	122	GGUUAUCGAUGACCUUACC	818
AUCACUCCCCUGUAGGAA	123	AUCACUCCCCUGUAGGAA	123	UUCCUCACAGGGGAGUGAU	819
UCACUCCCCUGUAGGAAC	124	UCACUCCCCUGUAGGAAC	124	GUUCCUCACAGGGGAGUGA	820
UGUCUUCACGCAGAAAGCG	125	UGUCUUCACGCAGAAAGCG	125	CGCUUUCUGCGUGAAGACA	821
UCACGCAGAAAGCGUUA	126	UCACGCAGAAAGCGUUA	126	CUAGACGCUUUCUGCGUGA	822
CACGCAGAAAGCGUUA	127	CACGCAGAAAGCGUUA	127	GCUAGACGCUUUCUGCGUG	823
GACCGGUCCUUUCUUGGA	128	GACCGGUCCUUUCUUGGA	128	UCCAAGAAAGGACCCCGUC	824
GAGGAACUACUGUCUUCAC	129	GAGGAACUACUGUCUUCAC	129	GUGAAGACAGUAGUUCUC	825
CUGUGAGGAACUACUGUCU	130	CUGUGAGGAACUACUGUCU	130	AGACAGUAGUUCUUCACAG	826
GGAACUACUGUCUUCACGC	131	GGAACUACUGUCUUCACGC	131	GCGUGAAGACAGUAGUUC	827
ACUCCCCUGUAGGAACUA	132	ACUCCCCUGUAGGAACUA	132	UAGUCCUCACAGGGGAGU	828
GUCUUCACGCAGAAAGCGU	133	GUCUUCACGCAGAAAGCGU	133	ACGCUUUCUGCGUGAAGAC	829
AGGAACUACUGUCUUCACG	134	AGGAACUACUGUCUUCACG	134	CGUGAAGACAGUAGUUCU	830
CCUGUGAGGAACUACUGUC	135	CCUGUGAGGAACUACUGUC	135	GACAGUAGUUCUUCACAGG	831
UGUGAGGAACUACUGUCU	136	UGUGAGGAACUACUGUCU	136	AAGACAGUAGUUCUUCACACA	832
UCUUCACGCAGAAAGCGUC	137	UCUUCACGCAGAAAGCGUC	137	GACGCUUUCUGCGUGAAGA	833
GAACUACUGUCUUCACGCA	138	GAACUACUGUCUUCACGCA	138	UGCGUGAAGACAGUAGUUC	834

CCCUGUGAGGAACUACUGU	139	CCCUGUGAGGAACUACUGU	139	ACAGUAGUCCUCACAGGG	835
CUUCACGCAGAAAGCGUCU	140	CUUCACGCAGAAAGCGUCU	140	AGACGCUUUCUGCGUGAAG	836
UGAGGAACUACUGUCUUA	141	UGAGGAACUACUGUCUUA	141	UGAAGACAGUAGUUCUCA	837
UGGCGUUAUAUGAGUGUC	142	UGGCGUUAUAUGAGUGUC	142	GACACUCAUACUAAACGCCA	838
CCCUGUGAGGAACUACUG	143	CCCUGUGAGGAACUACUG	143	CAGUAGUUCUCCUCACAGGGG	839
GUGAGGAACUACUGUCUUC	144	GUGAGGAACUACUGUCUUC	144	GAAGACAGUAGUUCUCCAC	840
GGCGUUAUAUGAGUGUCG	145	GGCGUUAUAUGAGUGUCG	145	CGACACUCAUACUAAACGCC	841
GCCGAGUAGUUGGUGUCG	146	GCCGAGUAGUUGGUGUCG	146	CGACCCAACACUACUCCGC	842
ACUGUCUUCACGCAGAAAG	147	ACUGUCUUCACGCAGAAAG	147	CUUUCUGCGUGAAGACAGU	843
UGGGUCGCGAAAGGCCUUG	148	UGGGUCGCGAAAGGCCUUG	148	CAAGGCCUUCGCGACCCCA	844
CUACUGUCUUCACGCAGAA	149	CUACUGUCUUCACGCAGAA	149	UUCUGCGUGAAGACAGUAG	845
CGAGUAGUGUUGGUGCGG	150	CGAGUAGUGUUGGUGCGG	150	CGCGACCCAACACUACUCCG	846
GUAGUGUUGGUGCGCGAAA	151	GUAGUGUUGGUGCGCGAAA	151	UUUCGCGACCCAACACUAC	847
UAAACCCUCAAGAAACACC	152	UAAACCCUCAAGAAACACC	152	GGUUUUUUCUUUGAGGUUUA	848
CCGAGUAGUGUUGGUGCG	153	CCGAGUAGUGUUGGUGCG	153	GCGACCCAACACUACUCCG	849
AGCCGAGUAGUGUUGGUG	154	AGCCGAGUAGUGUUGGUG	154	GACCCAACACUACUCCGCU	850
GUCGCGAAAGGCCUUGUGG	155	GUCGCGAAAGGCCUUGUGG	155	CCACAAGGCCUUCUCCGCAC	851
UAGUGUUGGUGCGCGAAAG	156	UAGUGUUGGUGCGCGAAAG	156	CUUUCGCGACCCAACACUA	852
CUAGCCGAGUAGUGUUGGG	157	CUAGCCGAGUAGUGUUGGG	157	CCCAACACUACUCCGCUAG	853
GAGUAGUGUUGGUGCGCGA	158	GAGUAGUGUUGGUGCGCGA	158	UCGCGACCCAACACUACUC	854
UCGCGAAAGGCCUUGUGGU	159	UCGCGAAAGGCCUUGUGGU	159	ACCACAAGGCCUUCUCCGGA	855
GCGUUAUAUAUGAGUGUCGU	160	GCGUUAUAUAUGAGUGUCGU	160	ACGACACUCAUACUAAACGC	856
UAGCCGAGUAGUGUUGGGU	161	UAGCCGAGUAGUGUUGGGU	161	ACCCAACACUACUCCGCUA	857
AACUACUGUCUUCACGCAG	162	AACUACUGUCUUCACGCAG	162	CUGCGUGAAGACAGUAGUU	858
CGCGAAAGGCCUUGUGGUA	163	CGCGAAAGGCCUUGUGGUA	163	UACCACAAGGCCUUCUCCGG	859
AGUGUUGGUGCGCGAAAGG	164	AGUGUUGGUGCGCGAAAGG	164	CCUUCGCGACCCCAACACU	860
GUUGGGUCGCGAAAGGCCU	165	GUUGGGUCGCGAAAGGCCU	165	AGGCCUUCGCGACCCCAAC	861
AGUAGUGUUGGUGCGCGAA	166	AGUAGUGUUGGUGCGCGAA	166	UUCGCGACCCCAACACUACU	862
UUGGGUCGCGAAAGGCCU	167	UUGGGUCGCGAAAGGCCU	167	AAGGCCUUCGCGACCCCA	863
UCCCCUGUGAGGAACUACU	168	UCCCCUGUGAGGAACUACU	168	AGUAGUUCUCCUCACAGGGGA	864
UACUGUCUUCACGCAGAAA	169	UACUGUCUUCACGCAGAAA	169	UUUCUGCGUGAAGACAGUA	865
GUGUUGGGUCGCGAAAGGC	170	GUGUUGGGUCGCGAAAGGC	170	GCCUUCGCGACCCCAACAC	866
ACUACUGUCUUCACGCAGA	171	ACUACUGUCUUCACGCAGA	171	UCUGCGUGAAGACAGUAGU	867
CUGUCUUCACGCAGAAAGC	172	CUGUCUUCACGCAGAAAGC	172	GCUUCUGCGUGAAGACAG	868
GGGUCGCGAAAGGCCUUGU	173	GGGUCGCGAAAGGCCUUGU	173	ACAAGGCCUUCUCCGACCC	869
CCUAAACCCUCAAGAAAAA	174	CCUAAACCCUCAAGAAAAA	174	UUUUUCUUUGAGGUUUAGG	870

GGUCGCGAAAGGCCUUGUG	175	GGUCGCGAAAGGCCUUGUG	175	CACAAGGCCUUUCGCGACC	871
CUAAACCCUCAAAGAAAC	176	CUAAACCCUCAAAGAAAC	176	GUUUUUUUUUGAGGUUUG	872
UGUUGGGUCGCGAAAGGCC	177	UGUUGGGUCGCGAAAGGCC	177	GGCCUUUCGCGACCCACA	873
CUCCCUUGUGAGGAACUAC	178	CUCCCUUGUGAGGAACUAC	178	GUAGUCCUCACAGGGGAG	874
UCCUAAACCUCAAAGAAA	179	UCCUAAACCUCAAAGAAA	179	UUUUCUUUGAGGUUUUGGA	875
ACCGGGUCCUUUCUUGGAU	180	ACCGGGUCCUUUCUUGGAU	180	AUCCAAGAAAGGACCCGGU	876
AUCCUAAACCUCAAAGAA	181	AUCCUAAACCUCAAAGAA	181	UUUUUUGAGGUUUAGGAUU	877
UCAUAGCCUGGAGAUUUGG	182	UCAUAGCCUGGAGAUUUGG	182	CCAAUUCUCCAGGCAUUGA	878
AUGCCUGGAGAUUUGGGCG	183	AUGCCUGGAGAUUUGGGCG	183	CGCCCAAUUCUCCAGGCAU	879
AAUGCCUGGAGAUUUGGGC	184	AAUGCCUGGAGAUUUGGGC	184	GCCCAAUUCUCCAGGCAUU	880
CCGACCUCAUGGGGUACAU	185	CCGACCUCAUGGGGUACAU	185	AUGACCCCAUGAGGUCGG	881
GCUCAAUGCCUGGAGAUUU	186	GCUCAAUGCCUGGAGAUUU	186	AAUUCUCCAGGCAUUGAGC	882
CUCAAUGCCUGGAGAUUUG	187	CUCAAUGCCUGGAGAUUUG	187	CAAUUCUCCAGGCAUUGAG	883
GCUAGCCGAGUAGUUGG	188	GCUAGCCGAGUAGUUGG	188	CCAACACUACUCGGCUAGC	884
CGCUCAAUGCCUGGAGAUU	189	CGCUCAAUGCCUGGAGAUU	189	AAUUCUCCAGGCAUUGAGCG	885
CAAUGCCUGGAGAUUUGGG	190	CAAUGCCUGGAGAUUUGGG	190	CCCAAUUCUCCAGGCAUUG	886
GCCGACCUCAUGGGGUACA	191	GCCGACCUCAUGGGGUACA	191	UGUACCCCAUGAGGUCGGC	887
AUCCUAAACCUCAAAGAAA	192	AUCCUAAACCUCAAAGAAA	192	UUUCUUUGAGGUUUAGGAU	888
AGAUUUGGGCGUGCCCCCG	193	AGAUUUGGGCGUGCCCCCG	193	CGGGGCGACGCCCAAUUCU	889
CCCGCUCAAUGCCUGGAGA	194	CCCGCUCAAUGCCUGGAGA	194	UCUCCAGGCAUUGAGCGGG	890
GAGAUUUGGGCGUGCCCCC	195	GAGAUUUGGGCGUGCCCCC	195	GGGGCACGCCCAAUUCUC	891
GGAGAUUUGGGCGUGCCCCC	196	GGAGAUUUGGGCGUGCCCCC	196	GGGGCACGCCCAAUUCUCC	892
GAUUUGGGCGUGCCCCCGC	197	GAUUUGGGCGUGCCCCCGC	197	GCGGGGCGACGCCCAAUUC	893
CCGCUCAAUGCCUGGAGAU	198	CCGCUCAAUGCCUGGAGAU	198	AUCUCCAGGCAUUGAGCGG	894
AGUACACCGGAUUUGCCAG	199	AGUACACCGGAUUUGCCAG	199	CUGGCAUUUCCGGUGUACU	895
UACACCGGAUUUGCCAGGA	200	UACACCGGAUUUGCCAGGA	200	UCCUGGCAUUUCCGGUGUA	896
GAGUACACCGGAUUUGCCA	201	GAGUACACCGGAUUUGCCA	201	UGGCAUUUCCGGUGUACUC	897
GUACACCGGAUUUGCCAGG	202	GUACACCGGAUUUGCCAGG	202	CCUGGCAUUUCCGGUGUAC	898
UUGCCGCGCAGGGGCCCA	203	UUGCCGCGCAGGGGCCCA	203	UGGGGCCCCUGCGCGGCAA	899
CUGGAGAUUUGGGCGUGCC	204	CUGGAGAUUUGGGCGUGCC	204	GGCACGCCCAAUUCUCCAG	900
GUUGCCGCGCAGGGGCCCC	205	GUUGCCGCGCAGGGGCCCC	205	GGGGCCCCUGCGCGGCAAC	901
GCCUGGAGAUUUGGGCGUG	206	GCCUGGAGAUUUGGGCGUG	206	CACGCCCAAUUCUCCAGGC	902
UGGAGAUUUGGGCGUGCCC	207	UGGAGAUUUGGGCGUGCCC	207	GGGCACGCCCAAUUCUCCA	903
CCUGGAGAUUUGGGCGUGC	208	CCUGGAGAUUUGGGCGUGC	208	GCACGCCCAAUUCUCCAGG	904
UGCUGCCGAGUAGUUG	209	UGCUGCCGAGUAGUUG	209	CAACACUACUCGGCUAGCA	905
UGCCUGGAGAUUUGGGCGU	210	UGCCUGGAGAUUUGGGCGU	210	ACGCCCAAUUCUCCAGGCA	906

CUGCUAGCCGAGUAGUGU	211	CUGCUAGCCGAGUAGUGU	211	AACACUACUGGCUAGCAG	907
ACUGCUAGCCGAGUAGUGU	212	ACUGCUAGCCGAGUAGUGU	212	ACACUACUGGCUAGCAGU	908
GACUGCUAGCCGAGUAGUG	213	GACUGCUAGCCGAGUAGUG	213	CACUACUGGCUAGCAGUC	909
AGACUGCUAGCCGAGUAGU	214	AGACUGCUAGCCGAGUAGU	214	ACUACUGGCUAGCAGUCU	910
ACCCGCUCAUUGCCUGGAG	215	ACCCGCUCAUUGCCUGGAG	215	CUCCAGGCAUUGAGCGGGU	911
AACCCGCUCAUUGCCUGGA	216	AACCCGCUCAUUGCCUGGA	216	UCCAGGCAUUGAGCGGGUU	912
UGCCGCGCAGGGGCCCCAG	217	UGCCGCGCAGGGGCCCCAG	217	CUGGGGCCCCUGCGCGGCA	913
AGGGCCCCAGGUUGGGUG	218	AGGGCCCCAGGUUGGGUG	218	CACCCAACCUUGGGCCCCU	914
GGCCCCCAGGUUGGGUGUG	219	GGCCCCCAGGUUGGGUGUG	219	CACACCCAACCUUGGGCCCC	915
CAGGGCCCCCAGGUUGGGU	220	CAGGGCCCCCAGGUUGGGU	220	ACCCAACCUUGGGCCCCUG	916
GGCCCCCAGGUUGGGUGGC	221	GGCCCCCAGGUUGGGUGGC	221	GCACACCCAACCUUGGGGCC	917
CGCAGGGCCCCCAGGUUGG	222	CGCAGGGCCCCCAGGUUGG	222	CCAACCUUGGGCCCCUGCG	918
UGGGCAGGAUGGCUCUGU	223	UGGGCAGGAUGGCUCUGU	223	ACAGGAGCCAUCUUGGCCA	919
GCCCCAGGUUGGGUGCG	224	GCCCCAGGUUGGGUGCG	224	CGCACACCCAACCUUGGGGC	920
GCAGGGCCCCCAGGUUGGG	225	GCAGGGCCCCCAGGUUGGG	225	CCCAACCUUGGGCCCCUGC	921
GGCAGGAUGGCUCUCUG	226	GGCAGGAUGGCUCUCUG	226	GACAGGAGCCAUCUUGCCC	922
GGGGCCCCAGGUUGGGUGU	227	GGGGCCCCAGGUUGGGUGU	227	ACACCCAACCUUGGGCCCC	923
GCCGCGCAGGGGCCCCAGG	228	GCCGCGCAGGGGCCCCAGG	228	CCUGGGGCCCUUGCGCGGC	924
GCGCAGGGGCCCCAGGUUG	229	GCGCAGGGGCCCCAGGUUG	229	CAACCUUGGGGCCCUUGCGC	925
CGCGCAGGGGCCCCAGGUU	230	CGCGCAGGGGCCCCAGGUU	230	AACCUUGGGGCCCUUGCGCG	926
CCGCGCAGGGGCCCCAGGU	231	CCGCGCAGGGGCCCCAGGU	231	ACCUUGGGGCCCUUGCGCGG	927
AGGACGACCGGGUCCUUC	232	AGGACGACCGGGUCCUUC	232	GAAAGGACCGGGUCCUCCU	928
CAGGACGACCGGGUCCUUC	233	CAGGACGACCGGGUCCUUC	233	AAAGGACCGGGUCCUCCUG	929
UGCCAGGACGACCGGGUCC	234	UGCCAGGACGACCGGGUCC	234	GGACCCGGUCCUCCUGGCA	930
AUUGCCAGGACGACCGGGU	235	AUUGCCAGGACGACCGGGU	235	ACCCGGUCCUCCUGGCAAU	931
AAUUGCCAGGACGACCGGG	236	AAUUGCCAGGACGACCGGG	236	CCCGGUCCUCCUGGCAAUU	932
UUGCCAGGACGACCGGGUC	237	UUGCCAGGACGACCGGGUC	237	GACCCGGUCCUCCUGGCAA	933
CCAGGACGACCGGGUCCUU	238	CCAGGACGACCGGGUCCUU	238	AAGGACCCGGUCCUCCUGG	934
GCCAGGACGACCGGGUCCU	239	GCCAGGACGACCGGGUCCU	239	AGGACCCGGUCCUCCUGGC	935
GAAUUGCCAGGACGACCGG	240	GAAUUGCCAGGACGACCGG	240	CCGGUCCUCCUGGCAAUUC	936
ACGACCGGGUCCUUCUUG	241	ACGACCGGGUCCUUCUUG	241	CAAGAAAGGACCCCGGUCGU	937
GACGACCGGGUCCUUCUUC	242	GACGACCGGGUCCUUCUUC	242	AAGAAAGGACCCCGGUGUC	938
CGACCGGGUCCUUCUUGG	243	CGACCGGGUCCUUCUUGG	243	CCAAGAAAGGACCCCGGUCG	939
GGACGACCGGGUCCUUCU	244	GGACGACCGGGUCCUUCU	244	AGAAAGGACCCCGGUCGUCC	940
CCGGAUUGCCAGGACGAC	245	CCGGAUUGCCAGGACGAC	245	GUCGUCCUGGCAAUUCCGG	941
ACACCGGAUUGCCAGGAC	246	ACACCGGAUUGCCAGGAC	246	GUCCUGGCAAUUCCGGUGU	942

ACCGAAUUGCCAGGACGA	247	ACCGAAUUGCCAGGACGA	247	UCGUCCUGGCAAUUCCGGU	943
CGAAUUGCCAGGACGACC	248	CGAAUUGCCAGGACGACC	248	GGUCGUCCUGGCAAUUCCG	944
GGAAUUGCCAGGACGACC	249	GGAAUUGCCAGGACGACC	249	CGUCGUCCUGGCAAUUCC	945
CACCGAAUUGCCAGGACG	250	CACCGAAUUGCCAGGACG	250	CGUCCUGGCAAUUCCGGUG	946
CCCAGGUUGGGUGGCGC	251	CCCAGGUUGGGUGGCGC	251	GCGCACACCCAAACCUGGG	947
GAUCGUUGGGAGUUUAC	252	GAUCGUUGGGAGUUUAC	252	GUAAACUCCACCAACGAUC	948
CAGUUCGUUGGGAGUUU	253	CAGUUCGUUGGGAGUUU	253	AAACUCCACCAACGAUCUG	949
AGAUUCGUUGGGAGUUUA	254	AGAUUCGUUGGGAGUUUA	254	UAAACUCCACCAACGAUCU	950
CCAGGUUGGGUGGCGCG	255	CCAGGUUGGGUGGCGCG	255	CGCGACACCCAAACCUGGG	951
CCAGGUUGGGUGGCGCGC	256	CCAGGUUGGGUGGCGCGC	256	GCGCACACCCCAACCUGG	952
AGGUUGGGUGGCGCGGA	257	AGGUUGGGUGGCGCGGA	257	UCGCGCGCACACCCCAACCU	953
CAGGUUGGGUGGCGCGCG	258	CAGGUUGGGUGGCGCGCG	258	CGCGCGCACACCCCAACCUG	954
GGUUGGGUGGCGCGGAC	259	GGUUGGGUGGCGCGGAC	259	GUCGCGCGCACACCCCAACC	955
GAAAAACCAACGUAACAC	260	GAAAAACCAACGUAACAC	260	GUGUACGUUUUGGUUUUUC	956
AGAAAAACCAACGUAACA	261	AGAAAAACCAACGUAACA	261	UGUUAACGUUUUGGUUUUUCU	957
AACCAACGUAACACCAAC	262	AACCAACGUAACACCAAC	262	GUUGGUUUACGUUUUGGUU	958
AAAGAAAAACCAACGUA	263	AAAGAAAAACCAACGUA	263	UUACGUUUUGGUUUUUUCUUU	959
AAAAACCAACGUAACACC	264	AAAAACCAACGUAACACC	264	GGUGUUAACGUUUUGGUUUUU	960
AAGAAAAACCAACGUAAC	265	AAGAAAAACCAACGUAAC	265	GUUACGUUUUGGUUUUUUCUU	961
CAAGAAAAACCAACGUA	266	CAAGAAAAACCAACGUA	266	UACGUUUUGGUUUUUUCUUUG	962
ACCCCGCGGUAGGUCGCG	267	ACCCCGCGGUAGGUCGCG	267	CGCGACCUACGCCGGGGGU	963
GACCCCGCGGUAGGUCGCG	268	GACCCCGCGGUAGGUCGCG	268	GCGACCUACGCCGGGGGUC	964
CGUAGUAUGAGUGUCGUG	269	CGUAGUAUGAGUGUCGUG	269	CACGACACUCUAUACUACG	965
GUUAGUAUGAGUGUCGUG	270	GUUAGUAUGAGUGUCGUG	270	GCACGACACUCUAUACUAC	966
UUAGUAUGAGUGUCGUGCA	271	UUAGUAUGAGUGUCGUGCA	271	UGCACGACACUCUAUACUAA	967
CCAAACGUAACACCAACCG	272	CCAAACGUAACACCAACCG	272	CGGUUGGUUUUACGUUUUGG	968
ACCAACGUAACACCAACCC	273	ACCAACGUAACACCAACCC	273	GGUUGGUUUUACGUUUUGGU	969
UUGGGCGUGCCCCCGCGAG	274	UUGGGCGUGCCCCCGCGAG	274	CUCGGGGGCGACGCCCAA	970
AUUUGGGCGUGCCCCCGCG	275	AUUUGGGCGUGCCCCCGCG	275	CGCGGGGCGACGCCCAAU	971
UUUGGGCGUGCCCCCGCGGA	276	UUUGGGCGUGCCCCCGCGGA	276	UCGCGGGGCGACGCCCAA	972
AAACCAACGUAACACCAA	277	AAACCAACGUAACACCAA	277	UUUGGUUUUACGUUUUGGUU	973
UGGCGUGCCCCCGCGGAGA	278	UGGCGUGCCCCCGCGGAGA	278	UCUCGGGGGCGACGCCCA	974
GUCAGAUUCGUUGGAGU	279	GUCAGAUUCGUUGGAGU	279	ACUCCACCAACGAUCUGAC	975
GUGUCGUGCAGCCUCCAGG	280	GUGUCGUGCAGCCUCCAGG	280	CCUGGAGGCGUGCAGCAC	976
GGUCAGAUUCGUUGGAG	281	GGUCAGAUUCGUUGGAG	281	CUCCACCAACGAUCUGACC	977
AGUGUCGUGCAGCCUCCAG	282	AGUGUCGUGCAGCCUCCAG	282	CUGGAGGCGUGCAGCACACU	978

GAGUGUCGUGCAGCCUCCA	283	GAGUGUCGUGCAGCCUCCA	283	UGGAGGUCGACGACACUC	979
UCGUAGACCGUGCACC AUG	284	UCGUAGACCGUGCACC AUG	284	CAUGGUGCACGGUCUACGA	980
GACCGUGCACC AUGAGCAC	285	GACCGUGCACC AUGAGCAC	285	GUGCUCAUGGUGCACGGUC	981
AGUAUGAGUGUCGUGCAGC	286	AGUAUGAGUGUCGUGCAGC	286	GCUGCACGACACUCAUACU	982
UAGUAUGAGUGUCGUGCAG	287	UAGUAUGAGUGUCGUGCAG	287	CUGCACGACACUCAUACUA	983
UCAGAUCGUUGGUGGAGUU	288	UCAGAUCGUUGGUGGAGUU	288	AACUCCACCAACGAUCUGA	984
AGACCGUGCACC AUGAGCA	289	AGACCGUGCACC AUGAGCA	289	UGCUC AUGGUGCACGGUCU	985
AAAACCAAACGUAAACACCA	290	AAAACCAAACGUAAACACCA	290	UGGUGUACGUUUUGGUUUU	986
GUAGACCGUGCACC AUGAG	291	GUAGACCGUGCACC AUGAG	291	CUCAUGGUGCACGGUCUAC	987
CUCGUAGACCGUGCACC AU	292	CUCGUAGACCGUGCACC AU	292	AUGGUGCACGGUCUACGAG	988
CGUAGACCGUGCACC AUGA	293	CGUAGACCGUGCACC AUGA	293	UCAUGGUGCACGGUCUACG	989
CCUGGGCUCAGCCCGGUA	294	CCUGGGCUCAGCCCGGUA	294	UACCCGGGUGAGCCCCAGG	990
UAGACCGUGCACC AUGAGC	295	UAGACCGUGCACC AUGAGC	295	GCUCAUGGUGCACGGUCUA	991
GGUCUCGUAGACCGUGCAC	296	GGUCUCGUAGACCGUGCAC	296	GUGCACGGUCUACGAGACC	992
UCUCGUAGACCGUGCACC A	297	UCUCGUAGACCGUGCACC A	297	UGGUGCACGGUCUACGAGA	993
GUCUCGUAGACCGUGCACC	298	GUCUCGUAGACCGUGCACC	298	GGUGCACGGUCUACGAGAC	994
UUGGGUAAGGUAUCGAUA	299	UUGGGUAAGGUAUCGAUA	299	UAUCGAUACCUUACCCAA	995
UCGCCGACCUC AUGGGUA	300	UCGCCGACCUC AUGGGUA	300	UACCCCAUGAGGUGCGGCGA	996
CCUCAAGAAACCAAC	301	CCUCAAGAAACCAAC	301	GUUUGUUUUUUUUUGAGG	997
GGCGUGCCCCCGCAGAC	302	GGCGUGCCCCCGCAGAC	302	GUCUCGCGGGGCACGCC	998
GGAUGAACCGGCUGAUAGC	303	GGAUGAACCGGCUGAUAGC	303	GCUAUCAGCCGGUUAUCC	999
UGGAUGAACCGGCUGAUAG	304	UGGAUGAACCGGCUGAUAG	304	CUAUCAGCCGGUUAUCCA	1000
CUCAAAGAAACCAACG	305	CUCAAAGAAACCAACG	305	CGUUUGUUUUUUUUUGAG	1001
AGGAAGACUUCGAGCGGU	306	AGGAAGACUUCGAGCGGU	306	ACGCUUCGGAAGUCUCCU	1002
UCAAGAAACCAACCGU	307	UCAAGAAACCAACCGU	307	ACGUUUGUUUUUUUUUGA	1003
GGAAGACUUCGAGCGGUC	308	GGAAGACUUCGAGCGGUC	308	GACCGCUCGGAAGUCUCC	1004
CGCCGACCUCAUGGGGUAC	309	CGCCGACCUCAUGGGGUAC	309	GUACCCCAUGAGGUGCGG	1005
CUUCCGAGCGGUCGAACC	310	CUUCCGAGCGGUCGAACC	310	GGUUGCGACCCGUCGGAAG	1006
GGCGUGCCCCCGCAGACU	311	GGCGUGCCCCCGCAGACU	311	AGUCUCGCGGGGCGACGCC	1007
UAUGAGUGUCGUGCAGCCU	312	UAUGAGUGUCGUGCAGCCU	312	AGGCUGCACGACACUCAUA	1008
UGCCCCCGCAGACUGCUA	313	UGCCCCCGCAGACUGCUA	313	UAGCAGUCUCGCGGGGCA	1009
CGAGACUGCUAGCCGAGUA	314	CGAGACUGCUAGCCGAGUA	314	UACUCGGCUAGCAGUCUG	1010
UGAGUGUCGUGCAGCCUCC	315	UGAGUGUCGUGCAGCCUCC	315	GGAGGCUGCACGACACUCA	1011
GCCCCCGCAGACUGCUAG	316	GCCCCCGCAGACUGCUAG	316	CUAGCAGUCUCGCGGGGCG	1012
GAGACUGCUAGCCGAGUAG	317	GAGACUGCUAGCCGAGUAG	317	CUACUCGGCUAGCAGUCUC	1013
CCCCCGCAGACUGCUAGC	318	CCCCCGCAGACUGCUAGC	318	GCUAGCAGUCUCGCGGGG	1014

CGCGAGACUGCUAGCCGAG	319	CGCGAGACUGCUAGCCGAG	319	CUCGGCUAGCAGUCUCGCG	1015
GUAUGAGUGUCGUCAGCC	320	GUAUGAGUGUCGUCAGCC	320	GGCUGCAGACACUCUAUC	1016
AUGAGUGUCGUCAGCCUC	321	AUGAGUGUCGUCAGCCUC	321	GAGGCUACACGACACUCAU	1017
GCGAGACUGCUAGCCGAGU	322	GCGAGACUGCUAGCCGAGU	322	ACUCGGCUAGCAGUCUCGCG	1018
CCCCGCGAGACUGCUAGCC	323	CCCCGCGAGACUGCUAGCC	323	GGCUAGCAGUCUCGCGGGG	1019
CCGCGAGACUGCUAGCCGA	324	CCGCGAGACUGCUAGCCGA	324	UCGGCUAGCAGUCUCGCGG	1020
CCCGCGAGACUGCUAGCCG	325	CCCGCGAGACUGCUAGCCG	325	CGGCUAGCAGUCUCGCGGG	1021
GCGUGCCCCCGCGAGACUG	326	GCGUGCCCCCGCGAGACUG	326	CAGUCUCGCGGGGGCAGCG	1022
GACCCCCCUCGCCGGAGA	327	GACCCCCCUCGCCGGAGA	327	UCUCCCGGAGGGGGGUC	1023
CGGUCCUUUCUUGGAUCA	328	CGGUCCUUUCUUGGAUCA	328	UGAUCCAAAGAAAGACCCG	1024
GUGCCCCCGCGAGACUCU	329	GUGCCCCCGCGAGACUCU	329	AGCAGUCUCGCGGGGCAC	1025
CGUGCCCCCGCGAGACUCG	330	CGUGCCCCCGCGAGACUCG	330	GCAGUCUCGCGGGGGCAGG	1026
UUCGCCGACCUCAUGGGGU	331	UUCGCCGACCUCAUGGGGU	331	ACCCAUAGAGUCGGCGAA	1027
CGCCACACGAGACGUCAAGU	332	CGCCACACGAGACGUCAAGU	332	ACUUGACGUCCUGUGGGCG	1028
GCCACACGAGACGUCAAGUU	333	GCCACACGAGACGUCAAGUU	333	AACUUGACGUCCUGUGGGC	1029
ACCCCCCUCGCCGGAGAG	334	ACCCCCCUCGCCGGAGAG	334	CUCUCCGGGAGGGGGGU	1030
GGACCCCCCUCGCCGGAG	335	GGACCCCCCUCGCCGGAG	335	CUCCCGGAGGGGGGUCC	1031
CCGGUCCUUUCUUGGAUC	336	CCGGUCCUUUCUUGGAUC	336	GAUCCAAAGAAAGACCCCG	1032
CAGGACCCCCCUCGCCGG	337	CAGGACCCCCCUCGCCGG	337	CCCGGAGGGGGGUCCUG	1033
AGGACGUCAAGUUCGCCGG	338	AGGACGUCAAGUUCGCCGG	338	CCCGGAAACUUGACGUCCU	1034
AGGACCCCCCUCGCCGGA	339	AGGACCCCCCUCGCCGGA	339	UCCCGGAGGGGGGUCCU	1035
CCACAGGACGUCAAGUUC	340	CCACAGGACGUCAAGUUC	340	GGAACUUGACGUCCUGUG	1036
CAGGACGUCAAGUUCGCCG	341	CAGGACGUCAAGUUCGCCG	341	CCGGAAACUUGACGUCCUG	1037
ACAGGACGUCAAGUUCGCC	342	ACAGGACGUCAAGUUCGCC	342	CGGAAACUUGACGUCCUGU	1038
CACAGGACGUCAAGUUC	343	CACAGGACGUCAAGUUC	343	GGAAACUUGACGUCCUGUG	1039
CAGUGGAUGAACCGGCUGA	344	CAGUGGAUGAACCGGCUGA	344	UCAGCCGGUUAUCCACUG	1040
GGGCUAGCCCCGGUACCC	345	GGGCUAGCCCCGGUACCC	345	GGUACCCGGGCGUGAGCCC	1041
CCGAGCGGUCGCAACCUCG	346	CCGAGCGGUCGCAACCUCG	346	CGAGGUUGCGACCGCUCGG	1042
CUGGGCUCAGCCCCGGUAC	347	CUGGGCUCAGCCCCGGUAC	347	GUACCCGGGCGUGAGCCCAG	1043
AGUGGAUGAACCGGCUGAU	348	AGUGGAUGAACCGGCUGAU	348	AUCAGCCGGUUAUCCACU	1044
UCCGAGCGGUCGCAACCUC	349	UCCGAGCGGUCGCAACCUC	349	GAGGUUGCGACCGCUCGGA	1045
UGGGCUCAGCCCCGGUACC	350	UGGGCUCAGCCCCGGUACC	350	GGUACCCGGGCGUGAGCCCA	1046
GGUACCCUUGGCCCCCUA	351	GGUACCCUUGGCCCCCUA	351	UAGAGGGGCCAAGGGUACC	1047
UUCGAGCGGUCGCAACCU	352	UUCGAGCGGUCGCAACCU	352	AGUUUGCGACCGCUCGGAA	1048
GGGUACCCUUGGCCCCCU	353	GGGUACCCUUGGCCCCCU	353	AGAGGGGCCCAAGGGUACCC	1049
GGGUCCUUUCUUGGAUCA	354	GGGUCCUUUCUUGGAUCA	354	UUGAUCCAAAGAAAGGACCC	1050

CCCACAGGACGUCAAGUUC	355	CCCACAGGACGUCAAGUUC	355	GAACUUGACGUCCUGUGG	1051
GGUUGCUCUUUCUUAUCU	356	GGUUGCUCUUUCUUAUCU	356	AGAUAGAGAAAGAGCAACC	1052
GUGGGCAGGAUGGCUCCUG	357	GUGGGCAGGAUGGCUCCUG	357	CAGGAGCCAUCCUGCCCAC	1053
GGUGGCAGGAUGGCUCCU	358	GGUGGCAGGAUGGCUCCU	358	AGGAGCCAUCCUGCCCCACC	1054
GUUGCUCUUUCUUAUCUU	359	GUUGCUCUUUCUUAUCUU	359	AAGAUAGAGAAAGAGCAAC	1055
GUGGAUGAACCGGCUGAUA	360	GUGGAUGAACCGGCUGAUA	360	UAUCAGCCGGUUAUCCAC	1056
CCAGGACCCCCCUCCCGG	361	CCAGGACCCCCCUCCCGG	361	CCGGAGGGGGGUGCCUGG	1057
GGUGGGCAGGAUGGCUCC	362	GGUGGGCAGGAUGGCUCC	362	GGAGCCAUCCUGCCCACCC	1058
CUUCACGGAGGCUAUGACU	363	CUUCACGGAGGCUAUGACU	363	AGUCAUAGCCUCCGUGAAG	1059
ACGCCGCCACAGGACGU	364	ACGCCGCCACAGGACGU	364	ACGUCCUGUGGGCGGCGGU	1060
UCCAGGACCCCCCUCCCG	365	UCCAGGACCCCCCUCCCG	365	CGGGAGGGGGGUGCCUGGA	1061
AUAUGAUGAUAACUGGUC	366	AUAUGAUGAUAACUGGUC	366	GACCAGUUAUCAUCAUAU	1062
UUCACGGAGGCUAUGACUA	367	UUCACGGAGGCUAUGACUA	367	UAGUCAUAGCCUCCGUGAA	1063
UCACGGAGGCUAUGACUAG	368	UCACGGAGGCUAUGACUAG	368	CUAGUCAUAGCCUCCGUGA	1064
AUGAACCGGCUGAUAAGCGU	369	AUGAACCGGCUGAUAAGCGU	369	ACGCUAUCAGCCGGUUAU	1065
GGGAUAUGAUGAUAACUG	370	GGGAUAUGAUGAUAACUG	370	CAGUUAUCAUCAUAUCC	1066
UGCAGUGGAUGAACCGGCU	371	UGCAGUGGAUGAACCGGCU	371	AGCCGGUUAUCCACUGCA	1067
GUGCAGUGGAUGAACCGGC	372	GUGCAGUGGAUGAACCGGC	372	GCCGGUUAUCCACUGCAC	1068
UGAACCGGCUGAUAAGCGUU	373	UGAACCGGCUGAUAAGCGUU	373	AACGCUAUCAGCCGGUUA	1069
GGUAUAUGAUGAUAACUGG	374	GGUAUAUGAUGAUAACUGG	374	CCAGUUAUCAUCAUAUCC	1070
GCUCUUUCUCUAUCUUCCU	375	GCUCUUUCUCUAUCUUCCU	375	AGGAAGAUAGAGAAAGAGC	1071
GGGGCGACACUCCACCAU	376	GGGGCGACACUCCACCAU	376	AUGGUGGAGUGUGCCCCC	1072
GAUGAACCGGCUGAUAAGCG	377	GAUGAACCGGCUGAUAAGCG	377	CGCUAUCAGCCGGUUAUC	1073
GAUAUGAUGAUGAUAACUGGU	378	GAUAUGAUGAUGAUAACUGGU	378	ACCAGUUAUCAUCAUAUC	1074
UGGGAUAUGAUGAUAACU	379	UGGGAUAUGAUGAUAACU	379	AGUUAUCAUCAUAUCCCA	1075
UUGCUCUUUCUCUAUCUU	380	UUGCUCUUUCUCUAUCUU	380	GAAGAUAGAGAAAGAGCAA	1076
UGGGGGCGACACUCCACCA	381	UGGGGGCGACACUCCACCA	381	UGGUGGAGUGUGCCCCCA	1077
UGCUCUUUCUCUAUCUUCC	382	UGCUCUUUCUCUAUCUUCC	382	GGAAGAUAGAGAAAGAGCA	1078
GGUCCUUUCUUGGAUCAAC	383	GGUCCUUUCUUGGAUCAAC	383	GUUGAUCCAAAGAAAGGACC	1079
AAGACUUCGAGCGGUCGC	384	AAGACUUCGAGCGGUCGC	384	GCGACCGCUCGGAAGUCUU	1080
AGCCCGGGUACCCUUGGCC	385	AGCCCGGGUACCCUUGGCC	385	GGCCAAGGGUACCCGGGCU	1081
UUUCUUGGAUCAACCCGCU	386	UUUCUUGGAUCAACCCGCU	386	AGCGGUUUGAUCCAAGAAA	1082
CAGCCCGGGUACCCUUGGC	387	CAGCCCGGGUACCCUUGGC	387	GCCAAGGGUACCCGGGCUG	1083
AGACUUCGAGCGGUCGCA	388	AGACUUCGAGCGGUCGCA	388	UGCGACCGCUCGGAAGUCU	1084
UUCUUGGAUCAACCCGCUC	389	UUCUUGGAUCAACCCGCUC	389	GAGCGGUUUGAUCCAAGAA	1085
CCCGGGUACCCUUGGCCCC	390	CCCGGGUACCCUUGGCCCC	390	GGGGCCAAAGGUACCCCGG	1086

GUCCUUUCUUGGAUCAACC	391	GUCCUUUCUUGGAUCAACC	391	GGUUGAUCCAAGAAAGGAC	1087
CUUUCUUGGAUCAACCCGC	392	CUUUCUUGGAUCAACCCGC	392	GCGGUUGAUCCAAGAAAG	1088
CCUUUCUUGGAUCAACCCG	393	CCUUUCUUGGAUCAACCCG	393	C GGUUGAUCCAAGAAAGG	1089
UCCUUUCUUGGAUCAACCC	394	UCCUUUCUUGGAUCAACCC	394	GGUUGAUCCAAGAAAGGA	1090
AAGUCCCGGGCGGUGGUC	395	AAGUCCCGGGCGGUGGUC	395	GACCACCGCCCGGGAACUU	1091
GCAGUGGAUGAACCGGCUG	396	GCAGUGGAUGAACCGGCUG	396	CAGCCGUUCAUCCACUGC	1092
CCGGUACCCUUGGCCCCU	397	CCGGUACCCUUGGCCCCU	397	AGGGCCCAAGGUAACCCGG	1093
AGUCCCGGGCGGUGGUCA	398	AGUCCCGGGCGGUGGUCA	398	UGACCACCGCCCGGGAACU	1094
CUUGGAUCAACCCGCUCAA	399	CUUGGAUCAACCCGCUCAA	399	UUGAGCGGGUUGAUCCAAG	1095
GGAUCAACCCGCUCAUGC	400	GGAUCAACCCGCUCAUGC	400	GCAUUGAGCGGGUUGAUCC	1096
ACUCCGAGCGGUCGCAAC	401	ACUCCGAGCGGUCGCAAC	401	GUUGCGACCGCUCGGGAAGU	1097
UCUUGGAUCAACCCGCUCA	402	UCUUGGAUCAACCCGCUCA	402	UGAGCGGGUUGAUCCAAGA	1098
UUGGAUCAACCCGCUCAAU	403	UUGGAUCAACCCGCUCAAU	403	AUUGAGCGGGUUGAUCCA	1099
AACCGCCGCCACAGGACG	404	AACCGCCGCCACAGGACG	404	CGUCCUGUGGGCGCGGUU	1100
GCGUGAACUAUGCAACAGG	405	GCGUGAACUAUGCAACAGG	405	CCUGUUUGCAUAGUUCACGC	1101
AUCAACCCGCUCAAUGCCU	406	AUCAACCCGCUCAAUGCCU	406	AGGCAUUGAGCGGGUUGAU	1102
GAUCAACCCGCUCAAUGCC	407	GAUCAACCCGCUCAAUGCC	407	GGCAUUGAGCGGGUUGAU	1103
CAACCGCUCUAUGCCUGG	408	CAACCGCUCUAUGCCUGG	408	CCAGGCAUUGAGCGGGUUG	1104
GCUUCGCCGACCCUCAUGGG	409	GCUUCGCCGACCCUCAUGGG	409	CCAUGAGGUCGGCGGAAGC	1105
GACUUCGGAGCGGUCGCAA	410	GACUUCGGAGCGGUCGCAA	410	UUGCGACCGCUCGGAAGUC	1106
UCAACCCGCUCAAUGCCUG	411	UCAACCCGCUCAAUGCCUG	411	CAGGCAUUGAGCGGGUUGA	1107
GGCUUCGCCGACCCUCAUGG	412	GGCUUCGCCGACCCUCAUGG	412	CCAUGAGGUCGGCGGAAGCC	1108
UGGAUCAACCCGCUCAAUG	413	UGGAUCAACCCGCUCAAUG	413	CAUUGAGCGGGUUGAUCCA	1109
CGGGCGGUGGUCAGAUCCGU	414	CGGGCGGUGGUCAGAUCCGU	414	ACGAUCUGACCACCGCCCG	1110
CUUGGCCCCUCUAUGGCAA	415	CUUGGCCCCUCUAUGGCAA	415	UUGCCAUAGAGGGGCCAAG	1111
CCGGCGGUGGUCAGAUCCG	416	CCGGCGGUGGUCAGAUCCG	416	CGAUCUGACCACCGCCCGG	1112
UGGGGUGGGCAGGAUGGCU	417	UGGGGUGGGCAGGAUGGCU	417	AGCCAUCUUGCCCAACCCCA	1113
GGAGUUUACCUUGUCCCGC	418	GGAGUUUACCUUGUCCCGC	418	GCGGCAACAGGUAAACUCC	1114
CCUUGGCCCCUCUAUGGCA	419	CCUUGGCCCCUCUAUGGCA	419	UGCCAUAAGAGGGGCCAAGG	1115
GUGGAGUUUACCUUGUCC	420	GUGGAGUUUACCUUGUCC	420	GGCAACAGGUAAACUCCAC	1116
GGUGAGUUUACCUUGUCC	421	GGUGAGUUUACCUUGUCC	421	GCAACAGGUAAACUCCACC	1117
UUCGGCGGCGGUGGUCAGA	422	UUCGGCGGCGGUGGUCAGA	422	UCUGACCACCGCCCGGGA	1118
UGAACUAUGCAACAGGGAA	423	UGAACUAUGCAACAGGGAA	423	UUCCCUGUUGCAUAGUUA	1119
AGUUUACCUUGUCCGCGC	424	AGUUUACCUUGUCCGCGC	424	GCGCGCAACAGGUAAACU	1120
GUGAACUAUGCAACAGGGA	425	GUGAACUAUGCAACAGGGA	425	UCCUGUUGCAUAGUUCAC	1121
UUACCUUGUCCGCGCAGG	426	UUACCUUGUCCGCGCAGG	426	CCUGCGCGCAACAGGUAA	1122

UCCCGGGGUGGUCAGAU	427	UCCCGGGGUGGUCAGAU	427	AUCUGACCACCGCCCGGA	1123
GUUCCCCGGGUGGUCAG	428	GUUCCCCGGGUGGUCAG	428	CUGACCACCGCCCGGAAC	1124
GCCCGGUACCCUUGGCC	429	GCCCGGUACCCUUGGCC	429	GGGCAAGGUAACCCGGGC	1125
AAGGAGUAAGGCGAAGG	430	AAGGAGUAAGGCGAAGG	430	CCUUCGCCUUAUCUCCUU	1126
AGGAGUAAGGCGAAGGC	431	AGGAGUAAGGCGAAGGC	431	GCCUUCGCCUUAUCUCCU	1127
GUUUAACUGUUGCCGCGA	432	GUUUAACUGUUGCCGCGA	432	UGCGGGCAACAGGUAAAC	1128
CUGUUGCCGCGAGGGCC	433	CUGUUGCCGCGAGGGCC	433	GGCCCCUGCGCGGCAACAG	1129
AACACCAACCGCCGCCAC	434	AACACCAACCGCCGCCAC	434	GUGGGCGCGGUUGGUGUU	1130
GAGUUUACUGUUGCCGCG	435	GAGUUUACUGUUGCCGCG	435	CGCGGCAACAGGUAAACUC	1131
UUUACCUUGUUGCCGCGAG	436	UUUACCUUGUUGCCGCGAG	436	CUGCGGGCAACAGGUAAA	1132
GGGUGGGCAGGAUGGCUC	437	GGGUGGGCAGGAUGGCUC	437	GAGCAUCCUGCCCAACCCC	1133
GAAAGAUUCCGAGCGGUCG	438	GAAAGAUUCCGAGCGGUCG	438	CGACCGCUCGGAAGUCUUC	1134
ACCUUGUUGCCGCGAGGGG	439	ACCUUGUUGCCGCGAGGGG	439	CCCCUGCGGCAACAGGU	1135
UACCUUGUUGCCGCGAGGG	440	UACCUUGUUGCCGCGAGGG	440	CCCUUGCGGGCAACAGGUA	1136
UACCUUUAACUGGGCAG	441	UACCUUUAACUGGGCAG	441	CUGCCCAGUUGAAGAGGUA	1137
CGUGAACUAUGCAACAGGG	442	CGUGAACUAUGCAACAGGG	442	CCCUUGCAUAGUUCACG	1138
ACACCAACCGCCGCCACA	443	ACACCAACCGCCGCCACA	443	UGUGGGCGGGUUGGUGU	1139
CCCGGGGUGGUCAGAU	444	CCCGGGGUGGUCAGAU	444	GAUCUGACCACCGCCCGG	1140
ACCUUUAACUGGGCAGU	445	ACCUUUAACUGGGCAGU	445	ACUGCCCAGUUGAAGAGGU	1141
CUUCGCCGACCUCAUGGG	446	CUUCGCCGACCUCAUGGG	446	CCCCAUGAGGUUGGGGAAG	1142
CCUGUUGCCGCGCAGGGC	447	CCUGUUGCCGCGCAGGGC	447	GCCCCUGCGCGCAACAGG	1143
CCAACCGCCGCCACAGGA	448	CCAACCGCCGCCACAGGA	448	UCCUGUGGGCGGGUUGG	1144
ACCAACCGCCGCCACAGG	449	ACCAACCGCCGCCACAGG	449	CCUGUGGGCGGGUUGGU	1145
UGGAGUUUACCUUGUUGCCG	450	UGGAGUUUACCUUGUUGCCG	450	CGGCAACAGGUAAACUCCA	1146
CACCAACCGCCGCCACAG	451	CACCAACCGCCGCCACAG	451	CUGUGGGCGGGUUGGUG	1147
CAACGUAACACCAACCGC	452	CAACGUAACACCAACCGC	452	GCGGUUGGUGUACGUUUG	1148
CAAGCGGAGACGCGUGGAG	453	CAAGCGGAGACGCGUGGAG	453	CUCCAGCCGUCUCCGCUUG	1149
ACGGAGGCUAUGACUAGGU	454	ACGGAGGCUAUGACUAGGU	454	ACCUAGUCAUAGCCUCGCU	1150
UACACCAACCGCCGCCCA	455	UACACCAACCGCCGCCCA	455	UGGCGGGCGGUUGGUGUUA	1151
AUCGUUGGUGGAGUUUACC	456	AUCGUUGGUGGAGUUUACC	456	GGUAAACUCCACCAACGAU	1152
GGGAGACAUUAUCACAGC	457	GGGAGACAUUAUCACAGC	457	GCUGUAUAUAUGUCUCCC	1153
AACCUUGGGAAGGCGACA	458	AACCUUGGGAAGGCGACA	458	UGUCGCCUCCACAGGUU	1154
GGGGAGACAUUAUACACA	459	GGGGAGACAUUAUACACA	459	UGUGAUUAUAUGUCUCCCC	1155
AACGUAACACCAACCGCCG	460	AACGUAACACCAACCGCCG	460	CGGCGUUGGUGUUAACGUU	1156
AAACGUAACACCAACCGCC	461	AAACGUAACACCAACCGCC	461	GGCGGUUGGUGUUAACGUU	1157
GGGGAGACAUUAUACACAG	462	GGGGAGACAUUAUACACAG	462	CUGUGAUUAUAUGUCUCCCC	1158

GAGUAGGCGAAGGCGU	463	GAGUAGGCGAAGGCGU	463	ACGCCUUCGCCUUAUCUC	1159
AAGCGAGACGCUGGAGC	464	AAGCGGAGACGCUGGAGC	464	GCUCCAGCCGUCUCCGCUU	1160
GUACCCUUGGCCCCUCUAU	465	GUACCCUUGGCCCCUCUAU	465	AUAGAGGGGCCAAGGGUAC	1161
CCUCCAGGACCCCCCUCC	466	CCUCCAGGACCCCCCUCC	466	GGAGGGGGGUCCUGGAGG	1162
CUCCAGGACCCCCCUCCC	467	CUCCAGGACCCCCCUCCC	467	GGGAGGGGGGUCCUGGAG	1163
UACCCUUGGCCCCUCUAUG	468	UACCCUUGGCCCCUCUAUG	468	CAUAGAGGGGCCAAGGGUA	1164
CAACCUUGGGAAGGCGAC	469	CAACCUUGGGAAGGCGAC	469	GUCGCCUUCACGAGGUUG	1165
CGGAGGCUAUGACUAGGUA	470	CGGAGGCUAUGACUAGGUA	470	UACCUAGUCAUAGCCUCCG	1166
GGAGAUAGGCGAAGGCG	471	GGAGAUAGGCGAAGGCG	471	CGCCUUCGCCUUAUCUCC	1167
AGAUGAAGCGAAGGCGUC	472	AGAUGAAGCGAAGGCGUC	472	GACGCCUUCGCCUUAUCU	1168
GUACACCAACCGCCGCC	473	GUACACCAACCGCCGCC	473	GGCGGGGUUGGUGUUAAC	1169
CGUACACCAACCGCCGCC	474	CGUACACCAACCGCCGCC	474	GGCGGGGUUGGUGUUAAC	1170
ACGUACACCAACCGCCGC	475	ACGUACACCAACCGCCGC	475	GCGCGGUUGGUGUUAACG	1171
CACGGAGGCUAUGACUAGG	476	CACGGAGGCUAUGACUAGG	476	CCUAGUCAUAGCCUCCGUG	1172
GUUGGUGAGUUUACCUGU	477	GUUGGUGAGUUUACCUGU	477	ACAGGUAAACUCCACCAAC	1173
CGUUGGUGAGUUUACCUG	478	CGUUGGUGAGUUUACCUG	478	CAGGUAAACUCCACCAACG	1174
ACCCUUGGCCCCUCUAUGG	479	ACCCUUGGCCCCUCUAUGG	479	CCAUAGAGGGGCCAAGGGU	1175
UUGGUGAGUUUACCUGUU	480	UUGGUGAGUUUACCUGUU	480	AACAGGUAAACUCCACCAA	1176
UGGUGGAGUUUACCUGUUG	481	UGGUGGAGUUUACCUGUUG	481	CAACAGGUAAACUCCACCA	1177
UCGUUGGUGAGUUUACCUC	482	UCGUUGGUGAGUUUACCUC	482	AGGUAAACUCCACCAACGA	1178
CGGUACCCUUGGCCCCUC	483	CGGUACCCUUGGCCCCUC	483	GAGGGCCCAAGGUACCCG	1179
GGCUCAGCCCGGUACCCU	484	GGCUCAGCCCGGUACCCU	484	AGGGUACCCGGGUGAGCC	1180
GAUCACUCCCCUGUGAGGA	485	GAUCACUCCCCUGUGAGGA	485	UCCUCACAGGGGAGUGAUC	1181
GGUGGUCAGAUCCGUUGGUG	486	GGUGGUCAGAUCCGUUGGUG	486	CACCAACGAUCUGACCCACC	1182
GAUGAAGGCGAAGGCGUCC	487	GAUGAAGGCGAAGGCGUCC	487	GGACGCCUUCGCCUUAUC	1183
AGGAUGGCUCCUGUCACCC	488	AGGAUGGCUCCUGUCACCC	488	GGGUGACAGGAGCCAUCCU	1184
CUCAGCCCGGGUACCCUUG	489	CUCAGCCCGGGUACCCUUG	489	CAAGGGUACCCGGGUGAG	1185
UCAGCCCGGGUACCCUUGG	490	UCAGCCCGGGUACCCUUGG	490	CCAAGGGUACCCGGGUGA	1186
AUGAAGGCGAAGGCGUCCA	491	AUGAAGGCGAAGGCGUCCA	491	UGGACGCCUUCGCCUUAUC	1187
CGGGGGAGACAUUAUCAC	492	CGGGGGAGACAUUAUCAC	492	GUGAUUAUUGUCCGCCCG	1188
CAGGAUGGCUCCUGUCACC	493	CAGGAUGGCUCCUGUCACC	493	GGUGACAGGAGCCAUCCUG	1189
UGAAGGCGAAGGCGUCCAC	494	UGAAGGCGAAGGCGUCCAC	494	GUGGACGCCUUCGCCUUA	1190
UGGUCAGAUCCGUUGGGA	495	UGGUCAGAUCCGUUGGGA	495	UCCACCAACGAUCUGACCA	1191
GCUCAGCCCGGGUACCCU	496	GCUCAGCCCGGGUACCCU	496	AAGGGUACCCGGGUGAGC	1192
GUGGUCAGAUCCGUUGGUG	497	GUGGUCAGAUCCGUUGGUG	497	CCACCAACGAUCUGACCCAC	1193
CAGCCUCCAGGACCCGCC	498	CAGCCUCCAGGACCCGCC	498	GGGGGGGUCCUGGAGGCGUG	1194

GGCGGUGGUCAGAUUG	499	GGCGGUGGUCAGAUUG	499	CAACGAUCUGACCACCGC	1195
GCCUCCAGACCCCCUC	500	GCCUCCAGACCCCCUC	500	GAGGGGGUCCUGGAGGC	1196
AACCGGUGAUAGCGUUCG	501	AACCGGUGAUAGCGUUCG	501	CGAACGUAUCAGCCGGUU	1197
AGCUCCAGGACCCCCCU	502	AGCUCCAGGACCCCCCU	502	AGGGGGGUCUGGAGGCU	1198
CGCUUCGCCACCUCAUG	503	CGCUUCGCCACCUCAUG	503	CAUGAGGUCGGCGAAGCCG	1199
GCGGAGACGGCUGGAGCGC	504	GCGGAGACGGCUGGAGCGC	504	GCGUCCAGCGGUCUCCGC	1200
UCAUGGGGUACAUCGCU	505	UCAUGGGGUACAUCGCU	505	AGCGAAUGUACCCCAUGA	1201
GAACCGGCUAGAGCGUUC	506	GAACCGGCUAGAGCGUUC	506	GAACGUAUCAGCCGGUUC	1202
GCGGUGGUCAGAUUGG	507	GCGGUGGUCAGAUUGG	507	CCAACGAUCUGACCACCGC	1203
GGCAGGAUGGCUCCUGCA	508	GGCAGGAUGGCUCCUGCA	508	UGACAGGAGCCAUCCUGCC	1204
GCAGGAUGGCUCCUGAC	509	GCAGGAUGGCUCCUGAC	509	GUGACAGGAGCCAUCCUGC	1205
AUUUGGGUAAGGUCAUCGA	510	AUUUGGGUAAGGUCAUCGA	510	UCGAUGACCUUACCCAAAU	1206
ACCGGUGAUAGCGUUCGC	511	ACCGGUGAUAGCGUUCGC	511	GCGAACGUAUCAGCCGGU	1207
CGGAGACGGCUGGAGCGCG	512	CGGAGACGGCUGGAGCGCG	512	GCGCUCAGCCGUCUCCG	1208
GCGGCUUCGCCAGCCUCAU	513	GCGGCUUCGCCAGCCUCAU	513	AUGAGGUCGGCGAAGCCGC	1209
AUUUGGGUAAGGUCAUCG	514	AUUUGGGUAAGGUCAUCG	514	CGAUGACCUUACCCAAAU	1210
GGCGGUGGUCAGAUUGU	515	GGCGGUGGUCAGAUUGU	515	AACGAUCUGACCACCGCC	1211
CAACCGCGCCACAGGAC	516	CAACCGCGCCACAGGAC	516	GUCUUGGGCGCGGUGU	1212
UGCGGCUUCGCCAGCCUCA	517	UGCGGCUUCGCCAGCCUCA	517	UGAGGUCGGCGAAGCCGCA	1213
CGGUGGUCAGAUUGGU	518	CGGUGGUCAGAUUGGU	518	ACCAACGAUCUGACCACCG	1214
UUUGGUGUGCGCGACUA	519	UUUGGUGUGCGCGACUA	519	UAGUCGCGCGCACACCCAA	1215
GUGUGCGCGACUAGGAA	520	GUGUGCGCGACUAGGAA	520	UUCCUAGUCGCGGCACAC	1216
GAUGGCUCCUGACCCCG	521	GAUGGCUCCUGACCCCG	521	CGGGUGACAGGAGCCAU	1217
GGAUGGCUCCUGACCCCG	522	GGAUGGCUCCUGACCCCG	522	GGGUGACAGGAGCCAUCC	1218
UGUGCGCGACUAGGAAG	523	UGUGCGCGACUAGGAAG	523	CUUCCUAGUCGCGGCACAC	1219
UGGGUGUGCGCGACUAG	524	UGGGUGUGCGCGACUAG	524	CUAGUCGCGCGCACACCCA	1220
GGUGUGCGCGACUAGGA	525	GGUGUGCGCGACUAGGA	525	UCCUAGUCGCGGCACACC	1221
GGGUGUGCGCGACUAGG	526	GGGUGUGCGCGACUAGG	526	CCUAGUCGCGGCACACCC	1222
CCCCGGGUGAGGUCGCUA	527	CCCCGGGUGAGGUCGCUA	527	UACGCGACCUACGCGGGG	1223
GAAGGCGACAACCUAUCC	528	GAAGGCGACAACCUAUCC	528	GGGAUAGGUUGUGCCUUC	1224
CCCGGCGUAGGUCGCUAA	529	CCCGGCGUAGGUCGCUAA	529	UUACGCGACCUACGCGGG	1225
AGCGGAGACGGCUGGAGCG	530	AGCGGAGACGGCUGGAGCG	530	GCGUCCAGCGGUCUCCGCU	1226
CCCCGGGUGAGGUCGCGU	531	CCCCGGGUGAGGUCGCGU	531	ACGCGACCUACGCGGGG	1227
AGGCGAAGGCUCCACAGU	532	AGGCGAAGGCUCCACAGU	532	ACUGUGGAGGCCUUCGCCU	1228
AAGGCGAAGGCUCCACAG	533	AAGGCGAAGGCUCCACAG	533	CUGUGGAGGCCUUCGCCU	1229
GUUGGGUGUGCGCGGACU	534	GUUGGGUGUGCGCGGACU	534	AGUCGCGCGCACACCCCAAC	1230

CUCAUGGGGUACAUIUCCGC	535	CUCAUGGGGUACAUIUCCGC	535	GCGGAUGUACCCCAUGAG	1231
GGAAGGCGACAACCUAUCC	536	GGAAGGCGACAACCUAUCC	536	GGAUAGGUUGUGCCCUUCC	1232
GCAAGUUCUUGCCGACGG	537	GCAAGUUCUUGCCGACGG	537	CCGUCCGCAAGGAACUUGC	1233
UGCAGCCUCCAGGACCCCC	538	UGCAGCCUCCAGGACCCCC	538	GGGGUCCUGGAGGCUUGCA	1234
GGACUGCACGAUGCUCGUG	539	GGACUGCACGAUGCUCGUG	539	CACGAGCAUCGUGCAGUCC	1235
GAAGCGAAGGCGUCCACA	540	GAAGCGAAGGCGUCCACA	540	UGUGGACGCCUUCGCCUUC	1236
GCAACCUUGUGGAAGCGGA	541	GCAACCUUGUGGAAGCGGA	541	UCGCCUCCACGAGGUUGC	1237
GACGCGGCUGUGCUUGGU	542	GACGCGGCUGUGCUUGGU	542	ACCAAGCACAGCCCGCGUC	1238
ACGCGGCUGUGCUUGGUA	543	ACGCGGCUGUGCUUGGUA	543	UACCAAGCACAGCCCGCGU	1239
GUGCAGCCUCCAGGACCC	544	GUGCAGCCUCCAGGACCC	544	GGGUCCUGGAGGCUGCAC	1240
GCAGCCUCCAGGACCCCC	545	GCAGCCUCCAGGACCCCC	545	GGGGGUCCUGGAGGCUGC	1241
CGCAACCUUGUGGAAGCG	546	CGCAACCUUGUGGAAGCG	546	CGCCUCCACGAGGUUGCG	1242
UGUCGUGCAGCCUCCAGGA	547	UGUCGUGCAGCCUCCAGGA	547	UCCUGGAGGCUGCACGACA	1243
AUGGCUUGGGAUUGAUGA	548	AUGGCUUGGGAUUGAUGA	548	UCAUCAUAUCCCAAGCCAU	1244
CUUGGGAUUGAUGAUGAA	549	CUUGGGAUUGAUGAUGAA	549	UUCAUCAUAUCCCAAG	1245
CCCUUGGCCCCUUAUGGC	550	CCCUUGGCCCCUUAUGGC	550	GCCAUJAGAGGGGCCAAGGG	1246
UGGCUUGGGAUUGAUGAU	551	UGGCUUGGGAUUGAUGAU	551	AUCAUCAUAUCCCAAGCCA	1247
CUGUGCAGUGGAUAACCG	552	CUGUGCAGUGGAUAACCG	552	CGGUUCAUCCACUGCACAG	1248
AUGACGCGGCUGUGCUUG	553	AUGACGCGGCUGUGCUUG	553	CAAGCACAGCCCGCGUCAU	1249
GCUUGGGAUUGAUGAUGA	554	GCUUGGGAUUGAUGAUGA	554	UCAUCAUCAUAUCCCAAGC	1250
UAUGACGCGGCUGUGCUU	555	UAUGACGCGGCUGUGCUU	555	AAGCACAGCCCGCGUCAUA	1251
UGACGCGGCUGUGCUUGG	556	UGACGCGGCUGUGCUUGG	556	CCAAGCACAGCCCGCGUCA	1252
GGCUUGGGAUUGAUGAUG	557	GGCUUGGGAUUGAUGAUG	557	CAUCAUCAUAUCCCAAGCC	1253
UGUGCAGUGGAUAACCGG	558	UGUGCAGUGGAUAACCGG	558	CCGUAUCAUCCACUGCACA	1254
GCUGUGCAGUGGAUAACC	559	GCUGUGCAGUGGAUAACC	559	GGUUCAUCCACUGCACAGC	1255
CUCUUAACUCGGGCAGUAA	560	CUCUUAACUCGGGCAGUAA	560	UUACUGCCAGUUUGAAGAG	1256
CCUCGUGGAAGGCGACAAC	561	CCUCGUGGAAGGCGACAAC	561	GUUUGCGCCUUCACAGAGG	1257
UGUGUCACCCAGACAGUCG	562	UGUGUCACCCAGACAGUCG	562	CGACUGUCUGGGUGACACA	1258
GGCGUGAAACUAUGCAACAG	563	GGCGUGAAACUAUGCAACAG	563	CUGUUGCAUAGUUCACGCC	1259
CGGCGUGAACUAUGCAACA	564	CGGCGUGAACUAUGCAACA	564	UGUUGCAUAGUUCACGCCG	1260
GUGUCACCCAGACAGUCGA	565	GUGUCACCCAGACAGUCGA	565	UCGACUGUCUGGGUGACAC	1261
CCUCUUAACUCGGGCAGUA	566	CCUCUUAACUCGGGCAGUA	566	UACUGCCAGUUGAAGAGG	1262
CGUGGAAGGCGACAACCUA	567	CGUGGAAGGCGACAACCUA	567	UAGGUUGUCGCCUUCACAG	1263
UCGUGGAAGGCGACAACCU	568	UCGUGGAAGGCGACAACCU	568	AGGUUGUCGCCUUCACAGA	1264
CGGCCUAGUUGGGCCCCCA	569	CGGCCUAGUUGGGCCCCCA	569	UGGGCCCCCAACUAGGCCG	1265
CGACUAGGAAGACUUCCGA	570	CGACUAGGAAGACUUCCGA	570	UCGGAAGUCUUCUAGUCG	1266

UUUGGUUAGGUCAUCGAU	571	UUUGGUUAGGUCAUCGAU	571	AUCGAUAGCCUUAACCCAAA	1267
GUGGAAGCGACAAACCUAU	572	GUGGAAGCGACAAACCUAU	572	AUAGGUUGUCGCCUUCAC	1268
ACCUCGUGGAAGCGACAA	573	ACCUCGUGGAAGCGACAA	573	UUUGGCCUUCACAGAGU	1269
GCGACUAGGAAGACUUCGG	574	GCGACUAGGAAGACUUCGG	574	CGGAAGUCUUCUAGUCGC	1270
GUCGUGCAGCCUCCAGGAC	575	GUCGUGCAGCCUCCAGGAC	575	GUCCUGGAGGCGUCCAGAC	1271
UAGGAAGACUUCGAGCGG	576	UAGGAAGACUUCGAGCGG	576	CCGCUCCGGAAGUCUUCUA	1272
ACGGCGUGAACUAUGCAAC	577	ACGGCGUGAACUAUGCAAC	577	GUUGCAUAGUUCACGCCGU	1273
CUCGUGGAAGCGACAACC	578	CUCGUGGAAGCGACAACC	578	GUUGUGCCUUCACCGAG	1274
GGUCGCAACCUCGUGAAG	579	GGUCGCAACCUCGUGAAG	579	CUUCCACGAGGUUGCGACC	1275
CGGUCGCAACCUCGUGAA	580	CGGUCGCAACCUCGUGAA	580	UUCACAGAGGUUGCGACCG	1276
GCGCGGACUAGGAAGACU	581	GCGCGGACUAGGAAGACU	581	AGUCUCCUAGUCGCGCGC	1277
GACGGCGUGAACUAUGCAA	582	GACGGCGUGAACUAUGCAA	582	UUGCAUAGUUCACGCCGUC	1278
UAGAUACUCCCCUGUGAG	583	UAGAUACUCCCCUGUGAG	583	CUCACAGGGAGUGAUCUA	1279
AGCGGUCGCAACCUCGUGG	584	AGCGGUCGCAACCUCGUGG	584	CCACGAGGUUGCGACCGCU	1280
UGGAAGGCGACAAACCUAUC	585	UGGAAGGCGACAAACCUAUC	585	GAUAGGUUGUCGCCUUCUA	1281
CAGCGGACUAGGAAGACUU	586	CAGCGGACUAGGAAGACUU	586	AAGUCUCCUAGUCGCGCG	1282
CUAGGAAGACUUCGAGCG	587	CUAGGAAGACUUCGAGCG	587	CGCUCGGAAGUCUUCUAG	1283
GUGCGGCGACUAGGAAGA	588	GUGCGGCGACUAGGAAGA	588	UCUUCUAGUCGCGCGCAC	1284
AGAUCACUCCCCUGUGAGG	589	AGAUCACUCCCCUGUGAGG	589	CCUCACAGGGAGUGAUCU	1285
UGCGGCGACUAGGAAGAC	590	UGCGGCGACUAGGAAGAC	590	GUCUUCUAGUCGCGCGCA	1286
AUAGAUACUCCCCUGUGA	591	AUAGAUACUCCCCUGUGA	591	UCACAGGGAGUGAUCUAU	1287
GAGCGGUGCAACCUCGUG	592	GAGCGGUGCAACCUCGUG	592	CACGAGGUUGCGACCGCUC	1288
CACGAACGACUGCUCCAAC	593	CACGAACGACUGCUCCAAC	593	GUUGGAGCAGUCGUUCGUG	1289
GGCAAGUUCUUGCCGACG	594	GGCAAGUUCUUGCCGACG	594	CGUCGGCAAGGAACUUGCC	1290
UCGUGCAGCCUCCAGGACC	595	UCGUGCAGCCUCCAGGACC	595	GGUCCUGGAGGCGUCACGA	1291
GUCACGAACGACUGCUCCA	596	GUCACGAACGACUGCUCCA	596	UGGAGCAGUCGUUCGUGAC	1292
GCGGUCGCAACCUCGUGGA	597	GCGGUCGCAACCUCGUGGA	597	UCCACGAGGUUGCGACCGC	1293
GCGGACUAGGAAGACUUC	598	GCGGACUAGGAAGACUUC	598	GAAGUCUUCUAGUCGCGC	1294
GCUAUGACGCGGCGUGUC	599	GCUAUGACGCGGCGUGUC	599	GCACAGCCCGCGUCAUAGC	1295
UCACGAACGACUGCUCCA	600	UCACGAACGACUGCUCCA	600	UUGGAGCAGUCGUUCGUGA	1296
UCGCAACCUCGUGGAAGGC	601	UCGCAACCUCGUGGAAGGC	601	GCCUCCACGAGGUUGCGA	1297
CGUGCAGCCUCCAGGACCC	602	CGUGCAGCCUCCAGGACCC	602	GGGUCCUGGAGGCGUCACG	1298
GUCGCAACCUCGUGGAAGG	603	GUCGCAACCUCGUGGAAGG	603	CCUCCACGAGGUUGCGAC	1299
ACUAGGAAGACUUCGAGC	604	ACUAGGAAGACUUCGAGC	604	GCUCGGAAGUCUUCUAGU	1300
CAGGACUAGGAAGACUUC	605	CAGGACUAGGAAGACUUC	605	GGAAGUCUUCUAGUCGCG	1301
UGGGCGAAGCACAUUGGA	606	UGGGCGAAGCACAUUGGA	606	UCCACAUGUGCUUCGCCCA	1302

CCUUGCCUACUUAUCCAUG	607	CCUUGCCUACUUAUCCAUG	607	CAUGGAUAGUAGGCAAGG	1303
GCCUCAGGAACUUGGGU	608	GCCUCAGGAACUUGGGU	608	ACCCAAAGUUUCCUGAGGC	1304
UGCUAUGACGCGGCGUG	609	UGCUAUGACGCGGCGUG	609	CACAGCCCGGUGCAUAGCA	1305
UCGUCUCGCCACCGCUAC	610	UCGUCUCGCCACCGCUAC	610	GUAGCGUGGCGAGCACGA	1306
UGCCUCAGGAAACUUGGG	611	UGCCUCAGGAAACUUGGG	611	CCCAAGUUUCCUGAGGCA	1307
UGUCUCGUGCCGACCCCG	612	UGUCUCGUGCCGACCCCG	612	CGGGUCGGGACGAGACA	1308
UGUGCGGCGAGGAGUUGG	613	UGUGCGGCGAGGAGUUGG	613	CCCAUCCUGCCGCCACA	1309
GUCGUGCUCGCCACCGCUA	614	GUCGUGCUCGCCACCGCUA	614	UAGCGUGGCGAGCACGAC	1310
GAUJUCCACUACGUGACGG	615	GAUJUCCACUACGUGACGG	615	CCGUCACGUAGUGGAAUC	1311
GGGCCUUGCCUACUUAUCC	616	GGGCCUUGCCUACUUAUCC	616	GGAUAGUAGGCAAGGCC	1312
GCCUUGCCUACUUAUCCA	617	GCCUUGCCUACUUAUCCA	617	AUGGAUAGUAGGCAAGGC	1313
GACUAGGAAGACUUCGAG	618	GACUAGGAAGACUUCGAG	618	CUCGGAAGUCUUCUAGUC	1314
GCGGGGAGACAUUAUCA	619	GCGGGGAGACAUUAUCA	619	UGAUUAUUGUCUCCCCGC	1315
CGAGCGGUCGCAACCUCGU	620	CGAGCGGUCGCAACCUCGU	620	ACGAGUUGCGACCCGUCG	1316
GGCCUUGCCUACUUAUCCA	621	GGCCUUGCCUACUUAUCCA	621	UGGAUAGUAGGCAAGGCC	1317
AUUUCCACUACGUGACGGG	622	AUUUCCACUACGUGACGGG	622	CCCGUCACGUAGUGGAAU	1318
GGACGUCAGUUCGCGGC	623	GGACGUCAGUUCGCGGC	623	GCCCGGAACUUGACGUCC	1319
GAGUGCUAUGACGCGGGCU	624	GAGUGCUAUGACGCGGGCU	624	AGCCCGGUCAUAGCACUC	1320
GACGUCAGUUCGCGGCG	625	GACGUCAGUUCGCGGCG	625	CGCCCGGAACUUGACGUC	1321
UCAGCGACGGUUCUUGGUC	626	UCAGCGACGGUUCUUGGUC	626	GACCAAGACCCGUCGUGA	1322
UCAAGUUCGCGGCGGUGG	627	UCAAGUUCGCGGCGGUGG	627	CCACCGCCCGGGAACUUGA	1323
UCAAGGAGAUAGAGCGAA	628	UCAAGGAGAUAGAGCGAA	628	UUCGCCUUCAUUCCUUGA	1324
CCUAUCCCCAAGGUCGCC	629	CCUAUCCCCAAGGUCGCC	629	GGCGAGCCUUGGGGAUAGG	1325
CUUGACCUACCUACAGAUCA	630	CUUGACCUACCUACAGAUCA	630	UGAUCUGAGGUAGGUCAAG	1326
UUUCCACUACGUGACGGGC	631	UUUCCACUACGUGACGGGC	631	GCCCGUACGUAGUGGAA	1327
AGUGCUAUGACGCGGGCUG	632	AGUGCUAUGACGCGGGCUG	632	CAGCCCGGUCAUAGCACU	1328
ACGUCAGUUCGCGGCGG	633	ACGUCAGUUCGCGGCGG	633	CCGCCCGGGAACUUGACGU	1329
UCUGGAGACAUCCGGCCAG	634	UCUGGAGACAUCCGGCCAG	634	CUGGCCCGAUGUCUCCAGA	1330
GGCGAAGCACAUUGUGGAA	635	GGCGAAGCACAUUGUGGAA	635	UUCACAUUGUCUUCGCC	1331
UUGACCUACCUACAGAUCAU	636	UUGACCUACCUACAGAUCAU	636	AUGAUCUGAGGUAGGUCAA	1332
CCAAGCGGAGACGGCUGGA	637	CCAAGCGGAGACGGCUGGA	637	UCCAGCCGUCUCCGCUUGG	1333
ACCAAGCGGAGACGGCUGG	638	ACCAAGCGGAGACGGCUGG	638	CCAGCCGUCUCCGCUUGGU	1334
GGUGGCUUCAUGCCUCAG	639	GGUGGCUUCAUGCCUCAG	639	CUGAGGCAUGAAGCCACCC	1335
GUCAAGUUCGCGGCGGUG	640	GUCAAGUUCGCGGCGGUG	640	CACCGCCCGGGAACUUGAC	1336
CUCAAGGAGAUAGAGCGA	641	CUCAAGGAGAUAGAGCGA	641	UCGCCUUCAUUCCUUGAG	1337
GACCAAGCGGAGACGGCUG	642	GACCAAGCGGAGACGGCUG	642	CAGCCGUCUCCGCUUGGUC	1338

UCCAGGUCGGGCUCAACCA	643	UCCAGGUCGGGCUCAACCA	643	UGGUUGAGCCCGACCUGGA	1339
CUCUUUCUCUAUCUCCUC	644	CUCUUUCUCUAUCUCCUC	644	GAGGAAGAUAGAGAAAGAG	1340
GUCUGGAGACAUCCGGCCA	645	GUCUGGAGACAUCCGGCCA	645	UGGCCCGAUGUCUCCAGAC	1341
GUUGUGACUUGGCCCCCGA	646	GUUGUGACUUGGCCCCCGA	646	UCGGGGGCCAAAGUCACAAC	1342
AGACCUGGCUCAGUCCAA	647	AGACCUGGCUCAGUCCAA	647	UUGGACUGGAGCCAGGUCU	1343
CUUGCCUACUUAUCCAUUG	648	CUUGCCUACUUAUCCAUUG	648	CCAUGGAAUAGUAGGCAAG	1344
CCCGGUUGCUCUUUCUCUA	649	CCCGGUUGCUCUUUCUCUA	649	UAGAGAAAGAGCAACCGG	1345
CUUUCUCUAUCUCCUCUU	650	CUUUCUCUAUCUCCUCUU	650	AAGAGGAAGAUAGAGAAAG	1346
AGGGUGGCUUCAUGCCUCA	651	AGGGUGGCUUCAUGCCUCA	651	UGAGGCAUGAAGCCACCCU	1347
AAGACCUGGCUCAGUCCA	652	AAGACCUGGCUCAGUCCA	652	UGGACUGGAGCCAGGUCUU	1348
CCGGUUGCUCUUUCUCUAU	653	CCGGUUGCUCUUUCUCUAU	653	AUAGAGAAAGAGCAACCGG	1349
CGGUUGCUCUUUCUCUAUC	654	CGGUUGCUCUUUCUCUAUC	654	GAUAGAGAAAGAGCAACCG	1350
UGGGGGAUUUCCACUACGU	655	UGGGGGAUUUCCACUACGU	655	ACGUAGUGGAAAUCCCCCA	1351
AUGUCACGAACGACUGCUC	656	AUGUCACGAACGACUGCUC	656	GAGCAGUCGUUCGUGACAU	1352
GGCCUAGUUGGGCCCCAC	657	GGCCUAGUUGGGCCCCAC	657	GUGGGCCCCAACUAGGCC	1353
UGGACCAAGCGGAGACGGC	658	UGGACCAAGCGGAGACGGC	658	GCCGUCUCCGCUUGGUCCA	1354
UUCACGUCGGGCUCAACC	659	UUCACGUCGGGCUCAACC	659	GGUUGAGCCCGACCUUGGAA	1355
AGCGGUCGAGUUCUUGGU	660	AGCGGUCGAGUUCUUGGU	660	ACCAGGAACUCGACCCGCU	1356
CAAGGAGAUAGAGCGGAAG	661	CAAGGAGAUAGAGCGGAAG	661	CUUCGCCUUCAUCUCCUUG	1357
CAUGUCACGAACGACUGCU	662	CAUGUCACGAACGACUGCU	662	AGCAGUCGUUCGUGACAU	1358
CAGCGGUCGAGUUCUUGG	663	CAGCGGUCGAGUUCUUGG	663	CCAGGAACUCGACCCGCGUG	1359
UUCACUACGUGACGGGCA	664	UUCACUACGUGACGGGCA	664	UGCCCGUCACGUAGUGGAA	1360
UAGGGUGGCUUCAUGCCUC	665	UAGGGUGGCUUCAUGCCUC	665	GAGGCAUGAAGCCACCCUA	1361
UCCAGGACUGCACGAUGCU	666	UCCAGGACUGCACGAUGCU	666	AGCAUCGUGCAGUCCUGGA	1362
UCCACUACGUGACGGGCAU	667	UCCACUACGUGACGGGCAU	667	AUGCCCGUCACGUAGUGGA	1363
AAUAGGGUGGCUUCAUGCC	668	AAUAGGGUGGCUUCAUGCC	668	GGCAUGAAGCCACCCUAUU	1364
GUUUUCACGGAGGCUAUGA	669	GUUUUCACGGAGGCUAUGA	669	UCAUAGCCUCCGUGAAGAC	1365
AUAGGGUGGCUUCAUGCCU	670	AUAGGGUGGCUUCAUGCCU	670	AGGCAUGAAGCCACCCUAU	1366
UCUUCACGGAGGCUAUGAC	671	UCUUCACGGAGGCUAUGAC	671	GUCAUAGCCUCCGUGAAGA	1367
AUGCCUCAGGAAACUUGGG	672	AUGCCUCAGGAAACUUGGG	672	CCCAAGUUUCCUGAGGCAU	1368
ACCGGACGUGCUCAAGGA	673	ACCGGACGUGCUCAAGGA	673	UCCUUGAGCACGUCGCCGU	1369
GGGCGUGUGCAGUGGAUGA	674	GGGCGUGUGCAGUGGAUGA	674	UCAUCCACUGCACAGCCCC	1370
AAGCUCCAGGACUGCACGA	675	AAGCUCCAGGACUGCACGA	675	UCGUGCAGUCCUGGAGCUU	1371
GCUCCAGGACUGCACGAUG	676	GCUCCAGGACUGCACGAUG	676	CAUCGUGCAGUCCUGGAGC	1372
UACCGGGACGUGCUCAAGG	677	UACCGGGACGUGCUCAAGG	677	CCUUGAGCACGUCGCCGUA	1373
GGGCGUGCAGUGGAUGAA	678	GGGCGUGCAGUGGAUGAA	678	UUCAUCCACUGCACAGCCCC	1374

CGUCAAGUCCCGGCGGU	679	CGUCAAGUCCCGGCGGU	679	ACCGCCCGGAACUUGACG	1375
UCAAUAGGUGGCUUCAUG	680	UCAAUAGGUGGCUUCAUG	680	CAUGAAGCCACCCUUAUGA	1376
AGUCUUCACGGAGGCUAUG	681	AGUCUUCACGGAGGCUAUG	681	CAUAGCCUCCGUGAAGACU	1377
GGACCAAGCGGAGACGGCU	682	GGACCAAGCGGAGACGGCU	682	AGCCGUCUCCGCUUGGUCC	1378
GGCUCCAGUCCAAGCUCCU	683	GGCUCCAGUCCAAGCUCCU	683	AGGAGCUUGGACUGGAGCC	1379
GGCUGUGCAGUGGAUAAAC	684	GGCUGUGCAGUGGAUAAAC	684	GUUCAUCCACUGCACAGCC	1380
CUCCAGGACUGCACGAUGC	685	CUCCAGGACUGCACGAUGC	685	GCAUCGUGCAGUCCUGGAG	1381
GAGUCUUCACGGAGGCUAU	686	GAGUCUUCACGGAGGCUAU	686	AUAGCCUCCGUGAAGACUC	1382
UGGCUCCAGUCCAAGCUCC	687	UGGCUCCAGUCCAAGCUCC	687	GGAGCUUGGACUGGAGCCA	1383
GGGGAUUUCCACUACGUGA	688	GGGGAUUUCCACUACGUGA	688	UCACGUAGUGGAAAUCCCC	1384
CAUGCCUCAGGAAACUUGG	689	CAUGCCUCAGGAAACUUGG	689	CCAAGUUUCCUGAGGCAUG	1385
AUCAAUAGGGUGGCUUCAU	690	AUCAAUAGGGUGGCUUCAU	690	AUGAAGCCACCCUUAUGAU	1386
GCGGGCCUUGCCUACUAAU	691	GCGGGCCUUGCCUACUAAU	691	AAUAGUAGGCAAGGCCCGC	1387
CCGGGACGUGCUCAGGAG	692	CCGGGACGUGCUCAGGAG	692	CUCCUUGAGCACGUCCCGG	1388
CCAUGGUGGGGAACUGGGC	693	CCAUGGUGGGGAACUGGGC	693	GCCAGUUCGCCACCAUGG	1389
CAUAAGGGUGGCUUCAUGC	694	CAUAAGGGUGGCUUCAUGC	694	GCAUGAAGCCACCCUUAUG	1390
AGCUCCAGGACUGCACGAU	695	AGCUCCAGGACUGCACGAU	695	AUCGUGCAGUCCUGGAGCU	1391
CGGGCCUUGCCUACUAAUUC	696	CGGGCCUUGCCUACUAAUUC	696	GAUAAGUAGGCAAGGCCCG	1392

The 3'-ends of the Upper sequence and the Lower sequence of the siNA construct can include an overhang sequence, for example about 1, 2, 3, or 4 nucleotides in length, preferably 2 nucleotides in length, wherein the overhanging sequence of the lower sequence is optionally complementary to a portion of the target sequence. The upper sequence is also referred to as the sense strand, whereas the lower sequence is also referred to as the antisense strand. The upper and lower sequences in the Table can further comprise a chemical modification having Formulae I-VII or any combination thereof.

Table III: HCV Synthetic Modified siNA constructs

HCV Target Sequence	SeqID	RPI#	Aliases	Sequence	SeqID
GGUCCUUUCUUGGAUCAACCCGC	1393	25237	HCV IRES Loop IIIb (sense)	B GGUCCUUUCUUGGAUCAACCC B	1413
GGUCCUUUCUUGGAUCAACCCGC	1393	25238	HCV IRES Loop (antisense)	B GGUUGAUCCCAAGAAAGGACC B	1414
GGUCCUUUCUUGGAUCAACCCGC	1393	25251	HCV IRES Loop (sense) Inverted Control	B CCCAACUAGGUUCUUUCCUGG B	1415
GGUCCUUUCUUGGAUCAACCCGC	1393	25252	HCV IRES Loop IIIb Inverted Control Compliment	B CCAGGAAAGAACCUAGUUGGG B	1416
GGUCCUUUCUUGGAUCAACCCGC	1393	25814	HCV IRES Loop IIIb +2U overhang sense	GGUCCUUUCUUGGAUCAACCCUU	1417
GGUCCUUUCUUGGAUCAACCCGC	1393	25815	HCV IRES Loop IIIb +2U overhang antisense	GGGUUGAUCCCAAGAAAGGACCUU	1418
GGUCCUUUCUUGGAUCAACCCGC	1393	25834	HCV IRES Loop IIIb +2U overhang sense	BGGUCCUUUCUUGGAUCAACCCUUB	1419
GGUCCUUUCUUGGAUCAACCCGC	1393	25835	HCV IRES Loop IIIb +2U overhang antisense	BGGGUUGAUCCCAAGAAAGGACCUUB	1420
UGCCCCGGGAGGUCUCGUAGACC	1394	28415	HCV-Luc:325U21 TT siRNA sense	CCCCGGGAGGUCUCGUAGATT	1421
UGCGGAACCGGUGAGUACACCGG	1395	28416	HCV-Luc:162U21 TT siRNA sense	CGGAACCGGUGAGUACACCTT	1422
GUGCCCCGGGAGGUCUCGUAGAC	1396	28417	HCV-Luc:324U21 TT siRNA sense	GCCCCGGGAGGUCUCGUAGTT	1423
GCGGAACCGGUGAGUACACCGGA	1397	28418	HCV-Luc:163U21 TT siRNA sense	GGAACCGGUGAGUACACCGTT	1424
UUUGGUACUGCCUGAUAGGGUG	1398	28419	HCV-Luc:294U21 TT siRNA sense	GUGGUACUGCCUGAUAGGGTT	1425
CUUGUGGUACUGCCUGAUAGGGU	1399	28420	HCV-Luc:293U21 TT siRNA sense	UGUGGUACUGCCUGAUAGGTT	1426
CCUUGUGGUACUGCCUGAUAGGG	1400	28421	HCV-Luc:292U21 TT siRNA sense	UUUGGUACUGCCUGAUAGTT	1427
UGCCCCGGGAGGUCUCGUAGACC	1394	28422	HCV-Luc:343L21 TT siRNA (325C) antisense	UCUACGAGACCUCCCCGGGTT	1428
UGCGGAACCGGUGAGUACACCGG	1395	28423	HCV-Luc:180L21 TT siRNA (162C) antisense	GGUGUACUCACCCGGUUCGTT	1429
GUGCCCCGGGAGGUCUCGUAGAC	1396	28424	HCV-Luc:342L21 TT siRNA (324C) antisense	CUACGAGACCUCCCCGGGCTT	1430
GCGGAACCGGUGAGUACACCGGA	1397	28425	HCV-Luc:181L21 TT siRNA (163C) antisense	CGGUGUACUCACCCGGUUCCTT	1431
UUUGGUACUGCCUGAUAGGGUG	1398	28426	HCV-Luc:312L21 TT siRNA (294C) antisense	CCCUAUCAGGCAGUACCACCTT	1432
CUUGUGGUACUGCCUGAUAGGGU	1399	28427	HCV-Luc:311L21 TT siRNA (293C) antisense	CCUUAUCAGGCAGUACCACATT	1433
CCUUGUGGUACUGCCUGAUAGGG	1400	28428	HCV-Luc:310L21 TT siRNA (292C) antisense	CUAUCAGGCAGUACCACAATT	1434
UGCCCCGGGAGGUCUCGUAGACC	1394	28429	HCV-Luc:325U21 TT siRNA Inv control	TTAGAUUGCUCUGGAGGGCCCC	1435

UGCGGAACCGGUGAGUACACCCGG	1395	28430	HCV-Luc:162U21 TT siRNA inv control	TTCACAUGAGUGGCAAGGC	1436
GUGCCCCGGAGGUCUCGUAGAC	1396	28431	HCV-Luc:324U21 TT siRNA inv control	TTGAUGCUCUGAGGGCCCCG	1437
GCGGAACCGGUGAGUACACCCGA	1397	28432	HCV-Luc:163U21 TT siRNA inv control	TTGCCACAUGAGUGGCCAAGG	1438
UUGUGGUACUGCCUGAUAGGGUG	1398	28433	HCV-Luc:294U21 TT siRNA inv control	TTGGGAUAGUCCGUCUAUGGUG	1439
CUUGUGGUACUGCCUGAUAGGGU	1399	28434	HCV-Luc:293U21 TT siRNA inv control	TTGGAUAGUCCGUCUAUGGUGU	1440
CCUUGUGGUACUGCCUGAUAGGG	1400	28435	HCV-Luc:292U21 TT siRNA inv control	TTGAUAGUCCGUCUAUGGUGUU	1441
UGCCCCGGGAGGUCUCGUAGACC	1394	28436	HCV-Luc:343L21 TT siRNA (325C) inv control	TTGGGGCCCUCCAGAGCAUCU	1442
UGCGGAACCGGUGAGUACACCCGG	1395	28437	HCV-Luc:180L21 TT siRNA (162C) inv control	TTGCCUUUGGCCACUCAUGUGG	1443
GUGCCCCGGGAGGUCUCGUAGAC	1396	28438	HCV-Luc:342L21 TT siRNA (324C) inv control	TTCGGGGCCCCUCCAGAGCAUC	1444
GCGGAACCGGUGAGUACACCCGA	1397	28439	HCV-Luc:181L21 TT siRNA (163C) inv control	TTCCUUUGGCCACUCAUGUGGC	1445
UUGUGGUACUGCCUGAUAGGGUG	1398	28440	HCV-Luc:312L21 TT siRNA (294C) inv control	TTACCAUGAGACGGACUAUCC	1446
CUUGUGGUACUGCCUGAUAGGGU	1399	28441	HCV-Luc:311L21 TT siRNA (293C) inv control	TTACCAUGAGACGGACUAUCC	1447
CCUUGUGGUACUGCCUGAUAGGG	1400	28442	HCV-Luc:310L21 TT siRNA (292C) inv control	TTAACACCAUGACGGACUAUC	1448
UGCGGAACCGGUGAGUACACCCGG	1395	29573	HCV-Luc:162U21 siRNA sense	CGGAACCGGUGAGUACACCCG	1449
GCGGAACCGGUGAGUACACCCGA	1397	29574	HCV-Luc:163U21 siRNA sense	GGAACCGGUGAGUACACCCGA	1450
CCUUGUGGUACUGCCUGAUAGGG	1400	29575	HCV-Luc:292U21 siRNA sense	UUGUGGUACUGCCUGAUAGGG	1451
CUUGUGGUACUGCCUGAUAGGGU	1399	29576	HCV-Luc:293U21 siRNA sense	UGUGGUACUGCCUGAUAGGGU	1452
UUGUGGUACUGCCUGAUAGGGUG	1398	29577	HCV-Luc:294U21 siRNA sense	GUGGUACUGCCUGAUAGGGUG	1453
GUGCCCCGGGAGGUCUCGUAGAC	1396	29578	HCV-Luc:324U21 siRNA sense	GCCCCGGGAGGUCUCGUAGAC	1454
UGCCCCGGGAGGUCUCGUAGACC	1394	29579	HCV-Luc:325U21 siRNA sense	CCCCGGGAGGUCUCGUAGACC	1455
UGCGGAACCGGUGAGUACACCCGG	1395	29580	HCV-Luc:182L21 siRNA (162C) antisense	GGUGUACUCACCGGUUCCGCA	1456
GCGGAACCGGUGAGUACACCCGA	1397	29581	HCV-Luc:183L21 siRNA (163C) antisense	CGGUGUACUCACCGGUUCCGC	1457
CCUUGUGGUACUGCCUGAUAGGG	1400	29582	HCV-Luc:312L21 siRNA (292C) antisense	CUAUCAGGCAGUACCAAGG	1458
CUUGUGGUACUGCCUGAUAGGGU	1399	29583	HCV-Luc:313L21 siRNA (293C) antisense	CCUAUCAGGCAGUACCAAG	1459
UUGUGGUACUGCCUGAUAGGGUG	1398	29584	HCV-Luc:314L21 siRNA (294C) antisense	CCCUAUCAGGCAGUACCAAA	1460
GUGCCCCGGGAGGUCUCGUAGAC	1396	29585	HCV-Luc:344L21 siRNA (324C) antisense	CUACGAGACCUCCCGGGGCAC	1461
UGCCCCGGGAGGUCUCGUAGACC	1394	29586	HCV-Luc:345L21 siRNA (325C) antisense	UCUACGAGACCUCCCGGGGCA	1462

UGCGGAACCGGUGAGUACACCGG	1395	29587	antisense	HCV-Luc:162U21 siRNA inv control	GGCCACAUGAGUGGCCAAGGC	1463
GCGGAACCGGUGAGUACACCGGA	1397	29588		HCV-Luc:163U21 siRNA inv control	AGGCCACAUGAGUGGCCAAGG	1464
CCUUGUGGUACUGCCUGAUAGGG	1400	29589		HCV-Luc:292U21 siRNA inv control	GGGAUAGUCCGUCUAUGGUGU	1465
CUUGUGGUACUGCCUGAUAGGGU	1399	29590		HCV-Luc:293U21 siRNA inv control	UGGGAUAGUCCGUCUAUGGUGU	1466
UUGUGGUACUGCCUGAUAGGGUG	1398	29591		HCV-Luc:294U21 siRNA inv control	GUGGGAUAGUCCGUCUAUGGUG	1467
GUGCCCCGGGAGGUCUCGUAGACC	1396	29592		HCV-Luc:324U21 siRNA inv control	CAGAUGCUCUGGAGGGCCCCCG	1468
UGCCCCGGGAGGUCUCGUAGACC	1394	29593		HCV-Luc:325U21 siRNA inv control	CCAGAUGCUCUGGAGGGCCCCC	1469
UGCGGAACCGGUGAGUACACCGG	1395	29594		HCV-Luc:182L21 siRNA (162C) inv control	ACGCCUUGGCCACUCUAUGGG	1470
GCGGAACCGGUGAGUACACCGGA	1397	29595		HCV-Luc:183L21 siRNA (163C) inv control	CGCCUUGGCCACUCUAUGGGC	1471
CCUUGUGGUACUGCCUGAUAGGG	1400	29596		HCV-Luc:312L21 siRNA (292C) inv control	GGAACACCAUGACGGACUAUC	1472
CUUGUGGUACUGCCUGAUAGGGU	1399	29597		HCV-Luc:313L21 siRNA (293C) inv control	GAACACCAUGACGGACUAUCC	1473
UUGUGGUACUGCCUGAUAGGGUG	1398	29598		HCV-Luc:314L21 siRNA (294C) inv control	AACACCAUGACGGACUAUCCC	1474
GUGCCCCGGGAGGUCUCGUAGACC	1396	29599		HCV-Luc:344L21 siRNA (324C) inv control	CACGGGGCCUCCAGAGCAUC	1475
UGCCCCGGGAGGUCUCGUAGACC	1394	29600		HCV-Luc:345L21 siRNA (325C) inv control	ACGGGGCCUCCAGAGCAUCU	1476
UGCCCCGGGAGGUCUCGUAGACC	1394	30051		HCV-Luc:325U21 siRNA 5' P=S + 3' univ. base 2 + 5'/3' invAba sense	BCsCsCsCsGsGGAGGUCUCGUAGAXXB	1477
UGCCCCGGGAGGUCUCGUAGACC	1394	30052		HCV-Luc:325U21 siRNA inv 5' P=S + 3' univ. base 2 + 5'/3' invAba	BAsGsAsUsGsCUCUGGAGGGCCCCXXB	1478
UGCCCCGGGAGGUCUCGUAGACC	1394	30053		HCV-Luc:345L21 siRNA (325C) 5' P=S + 3' univ. base 2 + 3' invAba antisense	UsCsUsAsCsGAGACCUCCCGGGXXB	1479
UGCCCCGGGAGGUCUCGUAGACC	1394	30054		HCV-Luc:345L21 siRNA (325C) inv 5' P=S + 3' univ. base 2 + 3' invAba	GsGsGsGsCsCCUCCAGAGCAUCUXXB	1480
UGCCCCGGGAGGUCUCGUAGACC	1394	30055		HCV-Luc:325U21 siRNA all Y P=S + 3' univ. base 2 + 5'/3' invAba sense	BCsCsCsCsGGGAGGUsCsUsCsGUsAGAXXB	1481
UGCCCCGGGAGGUCUCGUAGACC	1394	30056		HCV-Luc:325U21 siRNA inv all Y P=S + 3' univ. base 2 + 5'/3' invAba	BAGAUsgCsUsCsUsGGAGGGCsCsCsCsXXB	1482
UGCCCCGGGAGGUCUCGUAGACC	1394	30057		HCV-Luc:345L21 siRNA (325C) all Y P=S + 3' univ. base 2 + 3' invAba antisense	UsCsUsACsGAGACsCsUsCsCsGGGGXXB	1483
UGCCCCGGGAGGUCUCGUAGACC	1394	30058		HCV-Luc:345L21 siRNA (325C) inv all Y P=S + 3' univ. base 2 + 3' invAba	GGGGCsCsCsUsCsCsAGAGCsUsCsUsXXB	1484

UGCCCCGGGAGGUCUCGUAGACC	1394	30059	HCV-Luc:325U21 siRNA 4/3 P=S ends + all Y-2'F + 3' univ. base 2 + 5/3' invAba sense	BcscscscsGGGAGGucucGuAsGsAsXXB	1485
UGCCCCGGGAGGUCUCGUAGACC	1394	30060	HCV-Luc:325U21 siRNA inv 4/3 P=S ends + all Y-2'F + 3' univ. base 2 + 5/3' invAba	BAsGsAsusGcucuGGAGGGccscscsXXB	1486
UGCCCCGGGAGGUCUCGUAGACC	1394	30170	HCV-Luc:325U21 siRNA all Y-2'F + 3' univ. base 2 + 5/3' invAba sense	B cccccGGGAGGucucGuAGAXX B	1487
UGCCCCGGGAGGUCUCGUAGACC	1394	30171	HCV-Luc:325U21 siRNA inv all Y-2'F + 3' univ. base 2 + 5/3' invAba	B AGAuGcucuGGAGGGcccccXX B	1488
UGCCCCGGGAGGUCUCGUAGACC	1394	30172	HCV-Luc:345L21 siRNA (325C) all Y P=S + 3' univ. base 2 + 5/3' invAba antisense	UsCsUsACsGAGACsCsUsCsCsGGGGXX B	1489
UGCCCCGGGAGGUCUCGUAGACC	1394	30173	HCV-Luc:345L21 siRNA (325C) all Y-2'F antisense	ucuAcGAGAccucccGGGG	1490
UGCCCCGGGAGGUCUCGUAGACC	1394	30175	HCV-Luc:345L21 siRNA (325C) all Y-2'F + 3' univ. base 2 antisense	ucuAcGAGAccucccGGGGXX	1491
UGCCCCGGGAGGUCUCGUAGACC	1394	30176	HCV-Luc:345L21 siRNA (325C) inv all Y-2'F + 3' univ. base 2	GGGGcccccAGAGcAucXX	1492
UGCCCCGGGAGGUCUCGUAGACC	1394	30177	HCV-Luc:345L21 siRNA (325C) all Y-2'F + 3' univ. base 2 + 5/3' IB antisense	B ucuAcGAGAccucccGGGGXX B	1493
UGCCCCGGGAGGUCUCGUAGACC	1394	30178	HCV-Luc:325U21 siRNA all Y P=S + 3' univ. base 2 + 3' invAba sense	CsCsCsCsGGGAGGUsCsUsCsGsAGAXX B	1494
UGCCCCGGGAGGUCUCGUAGACC	1394	30417	HCV-Luc:325U21 siRNA w/IB sense	CCCCGGGAGGUCUCGUAGACC B	1495
UGCCCCGGGAGGUCUCGUAGACC	1394	30418	HCV-Luc:325U21 siRNA w/IB sense	B CCCCCGGGAGGUCUCGUAGACC B	1496
UGCCCCGGGAGGUCUCGUAGACC	1394	30419	HCV-Luc:345L21 siRNA (325C) w/IB antisense	UCUACGAGACCUCGCCGGGGCA B	1497
UGCCCCGGGAGGUCUCGUAGACC	1394	30420	HCV-Luc:345L21 siRNA (325C) w/IB antisense	B UCUACGAGACCUCGCCGGGGCA B	1498
UGCCCCGGGAGGUCUCGUAGACC	1394	30561	HCV-Luc:325U21 siRNA Y-2'OMe (stab06) + 5/3' invAba sense	BccccGGGAGGucucGuAGATTB	1499
UGCCCCGGGAGGUCUCGUAGACC	1394	30562	HCV-Luc:345L21 siRNA (325C) Y-2'F, R-2'OMe + TsT antisense	ucuAcGAGAccucccGGGGTsT	1500
AUAGUGGUCUGCGGAACCGGUGA	1401	30649	HCV-Luc:153U21 siRNA stab07 sense	B AGUGGucuGcGGAAccGGuTT B	1501
GUCUGCGGAACCGGUGAGUACAC	1402	30650	HCV-Luc:159U21 siRNA stab07 sense	B cuGcGGAAccGGGuGAGuAcTT B	1502
GCCUUGUGGUACUGCCUGAUAGG	1403	30651	HCV-Luc:291U21 siRNA stab07 sense	B cuuGuGGuAcuGccuGAuATT B	1503
UGUGGUACUGCCUGAUAGGGUGC	1404	30652	HCV-Luc:295U21 siRNA stab07 sense	B uGGuAcuGccuGAuAGGGGuTT B	1504
GUGGUACUGCCUGAUAGGGUGCU	1405	30653	HCV-Luc:296U21 siRNA stab07 sense	B GGuAcuGccuGAuAGGGGuTT B	1505
UGGUACUGCCUGAUAGGGUGCUU	1406	30654	HCV-Luc:297U21 siRNA stab07 sense	B GuAcuGccuGAuAGGGGuGcTT B	1506
GGUACUGCCUGAUAGGGUGCUUG	1407	30655	HCV-Luc:298U21 siRNA stab07 sense	B uAcuGccuGAuAGGGGuGcuTT B	1507

UACUGCCUGAUAGGGUGGUGCUUGCG	1408	30656	HCV-Luc:300U21 siRNA stab07 sense	B cuGccuGAuAGGGGuGcuuGTT B	1508
ACUGCCUGAUAGGGUGGUGCUUGCGA	1409	30657	HCV-Luc:301U21 siRNA stab07 sense	B uGccuGAuAGGGGuGcuuGcTT B	1509
UGCCUGAUAGGGUGGUGCUUGCGAGU	1410	30658	HCV-Luc:303U21 siRNA stab07 sense	B ccuGAuAGGGGuGcuuGcGATT B	1510
CUGAUAGGGUGGUGCUUGCGAGUGCC	1411	30659	HCV-Luc:306U21 siRNA stab07 sense	B GAuAGGGGuGcuuGcGAGuGTT B	1511
GUGCCCCGGAGGUGCUCGUAGAC	1396	30660	HCV-Luc:324U21 siRNA stab07 sense	B GccccGGAGGGuGucGuAGTT B	1512
AUAGUGGUGUCUGCGGAACCGGUGA	1401	30661	HCV-Luc:173L21 siRNA (153C) stab08 antisense	AccGGUuccGcAGAccAcuTsT	1513
GUCUGCGGAACCGGUGAGUACAC	1402	30662	HCV-Luc:179L21 siRNA (159C) stab08 antisense	GuAcucAccGGUuccGcAGTsT	1514
GCCUUGUGGUACUGCCUGAUAGG	1403	30663	HCV-Luc:311L21 siRNA (291C) stab08 antisense	uAucAGGcAGuAccAcAAAGTsT	1515
UGUGGUACUGCCUGAUAGGGUGC	1404	30664	HCV-Luc:315L21 siRNA (295C) stab08 antisense	AcccuAucAGGcAGuAccATsT	1516
GUGGUACUGCCUGAUAGGGUGCU	1405	30665	HCV-Luc:316L21 siRNA (296C) stab08 antisense	cAcccuAucAGGcAGuAccTsT	1517
UGGUACUGCCUGAUAGGGUGGCUU	1406	30666	HCV-Luc:317L21 siRNA (297C) stab08 antisense	GcAcccuAucAGGcAGuAcTsT	1518
GGUACUGCCUGAUAGGGUGGCUUG	1407	30667	HCV-Luc:318L21 siRNA (298C) stab08 antisense	AGcAcccuAucAGGcAGuAGTsT	1519
UACUGCCUGAUAGGGUGGUGCUUGCG	1408	30668	HCV-Luc:320L21 siRNA (300C) stab08 antisense	cAAGcAcccuAucAGGcAGTsT	1520
ACUGCCUGAUAGGGUGGUGCUUGCGA	1409	30669	HCV-Luc:321L21 siRNA (301C) stab08 antisense	GcAAGcAcccuAucAGGcAGTsT	1521
UGCCUGAUAGGGUGGUGCUUGCGAGU	1410	30670	HCV-Luc:323L21 siRNA (303C) stab08 antisense	ucGcAAGcAcccuAucAGGcAGTsT	1522
CUGAUAGGGUGGUGCUUGCGAGUGCC	1411	30671	HCV-Luc:326L21 siRNA (306C) stab08 antisense	cAcucGcAAGcAcccuAucTsT	1523
GUGCCCCGGAGGUGCUCGUAGAC	1396	30672	HCV-Luc:344L21 siRNA (324C) stab08 antisense	cuAcGAGAccuuccGGGcTsT	1524
AUAGUGGUGUCUGCGGAACCGGUGA	1401	30673	HCV-Luc:153U21 siRNA stab07 inv sense	B uGGccAAGGcGucGuGATT B	1525
GUCUGCGGAACCGGUGAGUACAC	1402	30674	HCV-Luc:159U21 siRNA stab07 inv sense	B cAuGAGuGGccAAGGcGucTT B	1526
GCCUUGUGGUACUGCCUGAUAGG	1403	30675	HCV-Luc:291U21 siRNA stab07 inv sense	B AuAGuccGucAuGGuGuucTT B	1527
UGUGGUACUGCCUGAUAGGGUGC	1404	30676	HCV-Luc:295U21 siRNA stab07 inv sense	B uGGGAuAGuccGucAuGGuTT B	1528
GUGGUACUGCCUGAUAGGGUGCU	1405	30677	HCV-Luc:296U21 siRNA stab07 inv sense	B GuGGGAuAGuccGucAuGGTT B	1529
UGGUACUGCCUGAUAGGGUGGCUU	1406	30678	HCV-Luc:297U21 siRNA stab07 inv sense	B cGuGGGAuAGuccGucAuGTT B	1530

GGUACUGCCUGAUAGGGUGUCUUG	1407	30679	HCV-Luc:298U21 siRNA stab07 inv sense	B ucGuGGGAuAGuccGucAuTT B	1531
UACUGCCUGAUAGGGUGUCUUGCG	1408	30680	HCV-Luc:300U21 siRNA stab07 inv sense	B GuucGuGGGAuAGuccGucTT B	1532
ACUGCCUGAUAGGGUGUCUUGCGA	1409	30681	HCV-Luc:301U21 siRNA stab07 inv sense	B cGuucGuGGGAuAGuccGuTT B	1533
UGCCUGAUAGGGUGUCUUGCGAGU	1410	30682	HCV-Luc:303U21 siRNA stab07 inv sense	B AGcGuucGuGGGAuAGuccTT B	1534
CUGAUAGGGUGUCUUGCGAGUGCC	1411	30683	HCV-Luc:306U21 siRNA stab07 inv sense	B GuGAGcGuucGuGGGAuAGTT B	1535
GUGCCCGGAGGUGUCUCGUAGAC	1396	30684	HCV-Luc:324U21 siRNA stab07 inv sense	B GAuGcucuGGAGGGccccGTT B	1536
AUAGUGGUCUGCGGAACCGGUGA	1401	30685	HCV-Luc:173L21 siRNA (153C) stab08 inv antisense	ucAccAGAcGccuuGGccATsT	1537
GUCUGCGGAACCGGUGAGUACAC	1402	30686	HCV-Luc:179L21 siRNA (159C) stab08 inv antisense	GAcGccuuGGccAcucAuGTsT	1538
GCCUUGUGGUACUGCCUGAUAGG	1403	30687	HCV-Luc:311L21 siRNA (291C) stab08 inv antisense	GAACAccAuGAcGGAcuAuTsT	1539
UGUGGUACUGCCUGAUAGGGUGC	1404	30688	HCV-Luc:315L21 siRNA (295C) stab08 inv antisense	AccAuGAcGGAcuAuccccATsT	1540
GUGGUACUGCCUGAUAGGGUGCU	1405	30689	HCV-Luc:316L21 siRNA (296C) stab08 inv antisense	ccAuGAcGGAcuAuccccAcTsT	1541
UGGUACUGCCUGAUAGGGUGCUU	1406	30690	HCV-Luc:317L21 siRNA (297C) stab08 inv antisense	cAuGAcGGAcuAuccccAcGTsT	1542
GGUACUGCCUGAUAGGGUGCUUG	1407	30691	HCV-Luc:318L21 siRNA (298C) stab08 inv antisense	AuGAcGGAcuAuccccAcGATsT	1543
UACUGCCUGAUAGGGUGCUUGCG	1408	30692	HCV-Luc:320L21 siRNA (300C) stab08 inv antisense	GAcGGAcuAuccccAcGAAcTsT	1544
ACUGCCUGAUAGGGUGCUUGCGA	1409	30693	HCV-Luc:321L21 siRNA (301C) stab08 inv antisense	AcGGAcuAuccccAcGAACGTsT	1545
UGCCUGAUAGGGUGCUUGCGAGU	1410	30694	HCV-Luc:323L21 siRNA (303C) stab08 inv antisense	GGAcuAuccccAcGAACGcuTsT	1546
CUGAUAGGGUGCUUGCGAGUGCC	1411	30695	HCV-Luc:326L21 siRNA (306C) stab08 inv antisense	cuAuccccAcGAACGcucAcTsT	1547
GUGCCCGGAGGUGUCUCGUAGAC	1396	30696	HCV-Luc:344L21 siRNA (324C) stab08 inv antisense	cGGGGccccuccAGAGcAucTsT	1548
UGCCCGGAGGUGUCUCGUAGACC	1394	31340	HCV-Luc:325U21 siRNA stab04 sense	B ccccGGGAGGucucGuAGATT B	1549
UGCCCGGAGGUGUCUCGUAGACC	1394	31341	HCV-Luc:325U21 siRNA inv stab04 sense	B AGAuGcucuGGAGGGccccTT B	1550
UGCCCGGAGGUGUCUCGUAGACC	1394	31342	HCV-Luc:345L21 siRNA (325C) stab05 antisense	ucuAcGAGAccucccccGGGTsT	1551
UGCCCGGAGGUGUCUCGUAGACC	1394	31343	HCV-Luc:345L21 siRNA (325C) inv	GGGGccccuccAGAGcAucTsT	1552

			stab05 antisense			
UGCCCCGGGAGGUCUCGUAGACC	1394	31344	HCV-Luc:325U21 siRNA stab07 sense	B ccccGGGAGGucucGuAGATT B		1553
UGCCCCGGGAGGUCUCGUAGACC	1394	31345	HCV-Luc:325U21 siRNA inv stab07 sense	B AGAuGcuuGGAGGGccccTT B		1554
UGCCCCGGGAGGUCUCGUAGACC	1394	31346	HCV-Luc:345L21 siRNA (325C) inv stab08 antisense	GGGGccccuccAGAGcAucUtsT		1555
UGCCCCGGGAGGUCUCGUAGACC	1394	31347	HCV-Luc:345L21 siRNA (325C) stab11 antisense	ucuAcGAGAccucccGGGGTsT		1556
UGCCCCGGGAGGUCUCGUAGACC	1394	31348	HCV-Luc:345L21 siRNA (325C) inv stab11 antisense	GGGGccccuccAGAGcAucUtsT		1557
AUAGUGGUCUGCGGAACCGGUGA	1401	31453	HCV-Luc:153U21 siRNA stab04 sense	B AGuGGucucGcGGAAccGGuTT B		1558
GUCUGCGGAACCGGUGAGUACAC	1402	31454	HCV-Luc:159U21 siRNA stab04 sense	B cuGcGGAAccGGuGAGuAcTT B		1559
AAAGGCCUUGUGGUACUGCCUGA	1412	31455	HCV-Luc:287U21 siRNA stab04 sense	B AGGccuuGuGGuAcuGccuTT B		1560
GCCUUGUGGUACUGCCUGAUAGG	1403	31456	HCV-Luc:291U21 siRNA stab04 sense	B cuuGuGGuAcuGccuGauATT B		1561
UGUGGUACUGCCUGAUAGGGUGC	1404	31457	HCV-Luc:295U21 siRNA stab04 sense	B uGGuAcuGccuGauAGGGuTT B		1562
GUGGUACUGCCUGAUAGGGUGCU	1405	31458	HCV-Luc:296U21 siRNA stab04 sense	B GGuAcuGccuGauAGGGuGTT B		1563
UGGUACUGCCUGAUAGGGUGCUU	1406	31459	HCV-Luc:297U21 siRNA stab04 sense	B GuAcuGccuGauAGGGuGcTT B		1564
GGUACUGCCUGAUAGGGUGCUUG	1407	31460	HCV-Luc:298U21 siRNA stab04 sense	B uAcuGccuGauAGGGuGcuTT B		1565
UACUGCCUGAUAGGGUGCUUGCG	1408	31461	HCV-Luc:300U21 siRNA stab04 sense	B cuGccuGauAGGGuGcuuGTT B		1566
ACUGCCUGAUAGGGUGCUUGCGA	1409	31462	HCV-Luc:301U21 siRNA stab04 sense	B uGccuGauAGGGuGcuuGcTT B		1567
UGCCUGAUAGGGUGCUUGCGAGU	1410	31463	HCV-Luc:303U21 siRNA stab04 sense	B ccuGauAGGGuGcuuGcGATT B		1568
CUGAUAGGGUGCUUGCGAGUGCC	1411	31464	HCV-Luc:306U21 siRNA stab04 sense	B GAuAGGGuGcuuGcGAGuGTT B		1569
AUAGUGGUCUGCGGAACCGGUGA	1401	31465	HCV-Luc:173L21 siRNA (153C) stab05 antisense	AccGGuuccGcAGAccAcuTsT		1570
GUCUGCGGAACCGGUGAGUACAC	1402	31466	HCV-Luc:179L21 siRNA (159C) stab05 antisense	GuAcucAccGGuuccGcAGTsT		1571
AAAGGCCUUGUGGUACUGCCUGA	1412	31467	HCV-Luc:307L21 siRNA (287C) stab05 antisense	AGGcAGuAccAcAAAGGccuTsT		1572
GCCUUGUGGUACUGCCUGAUAGG	1403	31468	HCV-Luc:311L21 siRNA (291C) stab05 antisense	uAucAGGcAGuAccAcAAAGTsT		1573
UGUGGUACUGCCUGAUAGGGUGC	1404	31469	HCV-Luc:315L21 siRNA (295C) stab05 antisense	AcccuAucAGGcAGuAccATsT		1574
GUGGUACUGCCUGAUAGGGUGCU	1405	31470	HCV-Luc:316L21 siRNA (296C) stab05 antisense	cAcccuAucAGGcAGuAccTsT		1575
UGGUACUGCCUGAUAGGGUGCUU	1406	31471	HCV-Luc:317L21 siRNA (297C) stab05 antisense	GcAcccuAucAGGcAGuAcTsT		1576
GGUACUGCCUGAUAGGGUGCUUG	1407	31472	HCV-Luc:318L21 siRNA (298C) stab05 antisense	AGcAcccuAucAGGcAGuATsT		1577
UACUGCCUGAUAGGGUGCUUGCG	1408	31473	HCV-Luc:320L21 siRNA (300C) stab05 antisense	cAAGcAcccuAucAGGcAGTsT		1578

ACUGCCUGAUAGGGUGCUUGCGA	1409	31474	HCV-Luc:321L21 siRNA (301C) stab05 antisense	GcAAGcAcccuAucAGGcATsT	1579
UGCCUGAUAGGGUGCUUGCGAGU	1410	31475	HCV-Luc:323L21 siRNA (303C) stab05 antisense	ucGcAAGcAcccuAucAGGTsT	1580
CUGAUAGGGUGCUUGCGAGUGCC	1411	31476	HCV-Luc:326L21 siRNA (306C) stab05 antisense	cAcucGcAAGcAcccuAucTsT	1581
AUAGUGGUCUGCGGAACCGGUGA	1401	31477	HCV-Luc:153U21 siRNA inv stab04 sense	B uGGccAAGGcGucGuGUGATT B	1582
GUCUGCGGAACCGGUGAGUACAC	1402	31478	HCV-Luc:159U21 siRNA inv stab04 sense	B cAuGAGuGGccAAGGcGucTT B	1583
AAAGGCCUUGUGGUACUGCCUGA	1412	31479	HCV-Luc:287U21 siRNA inv stab04 sense	B uccGucAuGGuGuuuccGGATT B	1584
GCCUUGUGGUACUGCCUGAUAGG	1403	31480	HCV-Luc:291U21 siRNA inv stab04 sense	B AuAGuccGucAuGGuGuuucTT B	1585
UGUGGUACUGCCUGAUAGGGUGC	1404	31481	HCV-Luc:295U21 siRNA inv stab04 sense	B uGGGAuAGuccGucAuGGuTT B	1586
GUGGUACUGCCUGAUAGGGUGCU	1405	31482	HCV-Luc:296U21 siRNA inv stab04 sense	B GuGGGAuAGuccGucAuGGTT B	1587
UGGUACUGCCUGAUAGGGUGCUU	1406	31483	HCV-Luc:297U21 siRNA inv stab04 sense	B cGuGGGAuAGuccGucAuGGTT B	1588
GGUACUGCCUGAUAGGGUGCUUG	1407	31484	HCV-Luc:298U21 siRNA inv stab04 sense	B ucGuGGGAuAGuccGucAuTT B	1589
UACUGCCUGAUAGGGUGCUUGCG	1408	31485	HCV-Luc:300U21 siRNA inv stab04 sense	B GuucGuGGGAuAGuccGucTT B	1590
ACUGCCUGAUAGGGUGCUUGCGA	1409	31486	HCV-Luc:301U21 siRNA inv stab04 sense	B cGuucGuGGGAuAGuccGuTT B	1591
UGCCUGAUAGGGUGCUUGCGAGU	1410	31487	HCV-Luc:303U21 siRNA inv stab04 sense	B AGcGuucGuGGGAuAGuccTT B	1592
CUGAUAGGGUGCUUGCGAGUGCC	1411	31488	HCV-Luc:306U21 siRNA inv stab04 sense	B GuGAGcGuucGuGGGAuAGTT B	1593
AUAGUGGUCUGCGGAACCGGUGA	1401	31489	HCV-Luc:173L21 siRNA (153C) inv stab05 antisense	ucAccAGAcGccuuGGccATsT	1594
GUCUGCGGAACCGGUGAGUACAC	1402	31490	HCV-Luc:179L21 siRNA (159C) inv stab05 antisense	GAcGccuuGGccAcucAuGTsT	1595
AAAGGCCUUGUGGUACUGCCUGA	1412	31491	HCV-Luc:307L21 siRNA (287C) inv stab05 antisense	uccGGAAcAccAuGAcGGATsT	1596
GCCUUGUGGUACUGCCUGAUAGG	1403	31492	HCV-Luc:311L21 siRNA (291C) inv stab05 antisense	GAACcAuGAcGGAcuAuTsT	1597
UGUGGUACUGCCUGAUAGGGUGC	1404	31493	HCV-Luc:315L21 siRNA (295C) inv stab05 antisense	AccAuGAcGGAcuAuucccATsT	1598
GUGGUACUGCCUGAUAGGGUGCU	1405	31494	HCV-Luc:316L21 siRNA (296C) inv stab05 antisense	ccAuGAcGGAcuAuucccAcTsT	1599

UGGUACUGCCUGAUAGGGUGCUU	1406	31495	HCV-Luc:317L21 siRNA (297C) inv stab05 antisense	cAuGAcGGAcuAuucccAcGTsT	1600
GGUACUGCCUGAUAGGGUGCUUG	1407	31496	HCV-Luc:318L21 siRNA (298C) inv stab05 antisense	AuGAcGGAcuAuucccAcGATsT	1601
UACUGCCUGAUAGGGUGCUUGCG	1408	31497	HCV-Luc:320L21 siRNA (300C) inv stab05 antisense	GAcGGAcuAuucccAcGAAcTsT	1602
ACUGCCUGAUAGGGUGCUUGCGA	1409	31498	HCV-Luc:321L21 siRNA (301C) inv stab05 antisense	AcGGAcuAuucccAcGAACGTsT	1603
UGCCUGAUAGGGUGCUUGCGAGU	1410	31499	HCV-Luc:323L21 siRNA (303C) inv stab05 antisense	GGAcuAuucccAcGAACGcuTsT	1604
CUGAUAGGGUGCUUGCGAGUGCC	1411	31500	HCV-Luc:326L21 siRNA (306C) inv stab05 antisense	cuAuucccAcGAACGcucAcTsT	1605

Uppercase = ribonucleotide
u,c = 2'-deoxy-2'-fluoro U, C
T = thymidine
B = inverted deoxy abasic
s = phosphorothioate linkage
A = deoxy Adenosine
G = deoxy Guanosine
X = universal base (5-nitroindole)
Z = universal base (3-nitropyrrole)

Table IV

Non-limiting examples of Stabilization Chemistries for chemically modified siNA constructs

Chemistry	pyrimidine	Purine	cap	p=S	Strand
“Stab 1”	Ribo	Ribo	-	5 at 5'-end 1 at 3'-end	S/AS
“Stab 2”	Ribo	Ribo	-	All linkages	Usually AS
“Stab 3”	2'-fluoro	Ribo	-	4 at 5'-end 4 at 3'-end	Usually S
“Stab 4”	2'-fluoro	Ribo	5' and 3'- ends	-	Usually S
“Stab 5”	2'-fluoro	Ribo	-	1 at 3'-end	Usually AS
“Stab 6”	2'-O-Methyl	Ribo	5' and 3'- ends	-	Usually S
“Stab 7”	2'-fluoro	2'-deoxy	5' and 3'- ends	-	Usually S
“Stab 8”	2'-fluoro	2'-O-Methyl	-	1 at 3'-end	Usually AS
“Stab 9”	Ribo	Ribo	5' and 3'- ends	-	Usually S
“Stab 10”	Ribo	Ribo	-	1 at 3'-end	Usually AS
“Stab 11”	2'-fluoro	2'-deoxy	-	1 at 3'-end	Usually AS

CAP = any terminal cap, see for example Figure 10.

All Stab 1-11 chemistries can comprise 3'-terminal thymidine (TT) residues

All Stab 1-11 chemistries typically comprise 21 nucleotides, but can vary as described herein.

S = sense strand

AS = antisense strand

Table V**A. 2.5 μ mol Synthesis Cycle ABI 394 Instrument**

Reagent	Equivalents	Amount	Wait Time* DNA	Wait Time* 2'-O-methyl	Wait Time*RNA
Phosphoramidites	6.5	163 μ L	45 sec	2.5 min	7.5 min
S-Ethyl Tetrazole	23.8	238 μ L	45 sec	2.5 min	7.5 min
Acetic Anhydride	100	233 μ L	5 sec	5 sec	5 sec
N-Methyl Imidazole	186	233 μ L	5 sec	5 sec	5 sec
TCA	176	2.3 mL	21 sec	21 sec	21 sec
Iodine	11.2	1.7 mL	45 sec	45 sec	45 sec
Beaucage	12.9	645 μ L	100 sec	300 sec	300 sec
Acetonitrile	NA	6.67 mL	NA	NA	NA

B. 0.2 μ mol Synthesis Cycle ABI 394 Instrument

Reagent	Equivalents	Amount	Wait Time* DNA	Wait Time* 2'-O-methyl	Wait Time*RNA
Phosphoramidites	15	31 μ L	45 sec	233 sec	465 sec
S-Ethyl Tetrazole	38.7	31 μ L	45 sec	233 min	465 sec
Acetic Anhydride	655	124 μ L	5 sec	5 sec	5 sec
N-Methyl Imidazole	1245	124 μ L	5 sec	5 sec	5 sec
TCA	700	732 μ L	10 sec	10 sec	10 sec
Iodine	20.6	244 μ L	15 sec	15 sec	15 sec
Beaucage	7.7	232 μ L	100 sec	300 sec	300 sec
Acetonitrile	NA	2.64 mL	NA	NA	NA

C. 0.2 μ mol Synthesis Cycle 96 well Instrument

Reagent	Equivalents:DNA/ 2'-O-methyl/Ribo	Amount: DNA/2'-O- methyl/Ribo	Wait Time* DNA	Wait Time* 2'-O- methyl	Wait Time* Ribo
Phosphoramidites	22/33/66	40/60/120 μ L	60 sec	180 sec	360sec
S-Ethyl Tetrazole	70/105/210	40/60/120 μ L	60 sec	180 min	360 sec
Acetic Anhydride	265/265/265	50/50/50 μ L	10 sec	10 sec	10 sec
N-Methyl Imidazole	502/502/502	50/50/50 μ L	10 sec	10 sec	10 sec
TCA	238/475/475	250/500/500 μ L	15 sec	15 sec	15 sec
Iodine	6.8/6.8/6.8	80/80/80 μ L	30 sec	30 sec	30 sec
Beaucage	34/51/51	80/120/120	100 sec	200 sec	200 sec
Acetonitrile	NA	1150/1150/1150 μ L	NA	NA	NA

- 5
- Wait time does not include contact time during delivery.
 - Tandem synthesis utilizes double coupling of linker molecule

CLAIMS

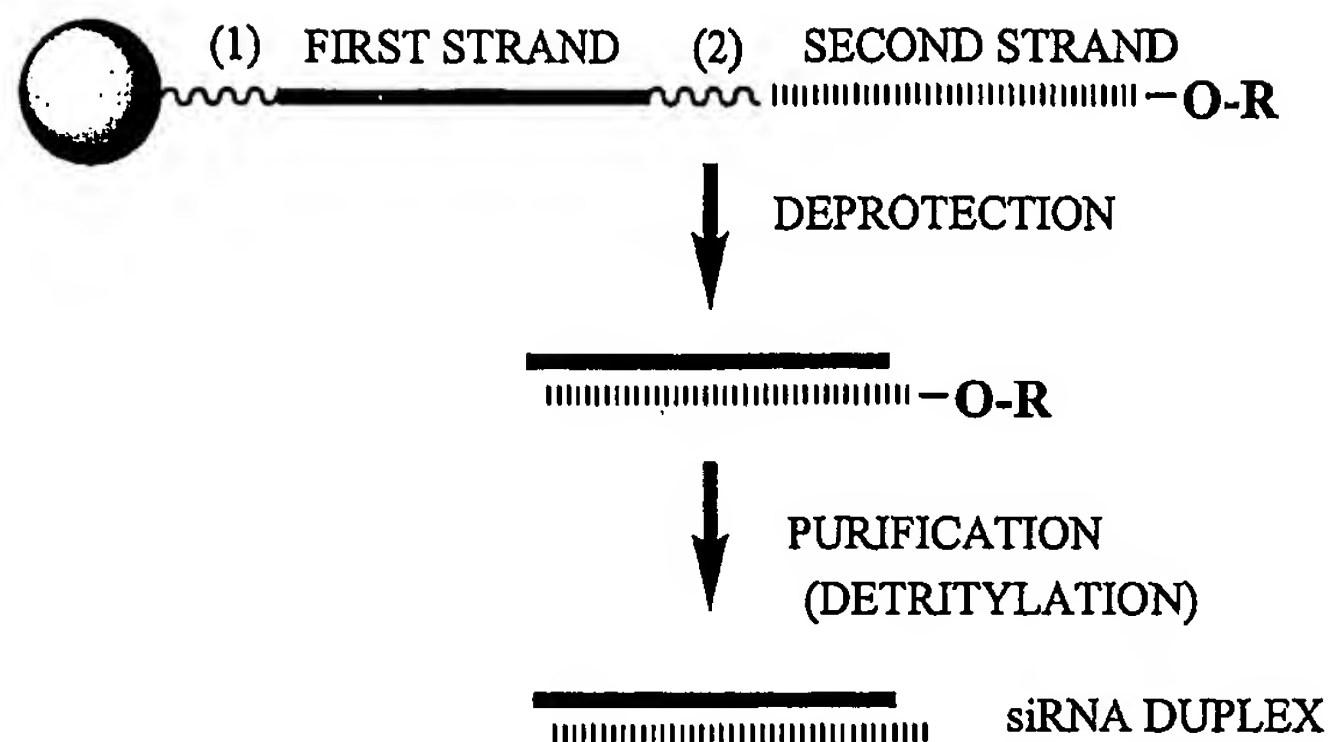
What we claim is:

1. A double-stranded short interfering nucleic acid (siNA) molecule that inhibits replication of a hepatitis C virus (HCV), wherein one of the strands of said double-stranded siNA molecule is an antisense strand which comprises a nucleotide sequence that is complementary to the nucleotide sequence of an HCV RNA or a portion thereof and the other strand is a sense strand which comprises a nucleotide sequence that is complementary to the nucleotide sequence of the antisense strand, and wherein a majority of the pyrimidine nucleotides present in said double-stranded siNA molecule comprises a sugar modification.
2. The siNA molecule of claim 1, wherein the HCV RNA comprises HCV minus strand RNA.
3. The siNA molecule of claim 1, wherein the HCV RNA comprises HCV plus strand RNA.
4. The siNA molecule of claim 1, wherein the siNA molecule comprises no ribonucleotides.
5. The siNA molecule of claim 1, wherein the siNA molecule comprises ribonucleotides.
6. The siNA molecule of claim 1, wherein all the pyrimidine nucleotides in the siNA molecule comprise sugar modifications.
7. The siNA molecule of claim 6, wherein the modified pyrimidine nucleotides are selected from 2'-deoxy-pyrimidine, 2'-O-alkyl pyrimidine, 2'-C-alkyl pyrimidine, 2'-deoxy-2'-fluoro-pyrimidine, 2'-amino pyrimidine, 2'-methoxy-ethoxy pyrimidine, and 2'-O, 4'-C-methylene pyrimidine nucleotides, alone or in any combination thereof.
8. The siNA molecule of claim 7, wherein the 2'-O-alkyl pyrimidine nucleotide is 2'-O-methyl or 2'-O-allyl.
9. The siNA molecule of claim 1, wherein the nucleotide sequence of the antisense strand of the double-stranded siNA molecule is complementary to an RNA encoding an HCV protein or a fragment thereof.

10. The siNA molecule of claim 1, wherein each strand of the siNA molecule comprises about 19 to about 29 nucleotides, and wherein each strand comprises at least about 19 nucleotides that are complementary to the nucleotides of the other strand.
- 5 11. The siNA molecule of claim 1, wherein said siNA molecule is assembled from two oligonucleotide fragments, wherein one fragment comprises the nucleotide sequence of the antisense strand of the siNA molecule and the second fragment comprises the nucleotide sequence of the sense strand of the siNA molecule.
12. The siNA molecule of claim 1, wherein the sense strand is connected to the antisense strand via a linker molecule.
- 10 13. The siNA molecule of claim 12, wherein said linker molecule is a polynucleotide linker.
14. The siNA molecule of claim 12, wherein said linker molecule is a non-nucleotide linker.
- 15 15. The siNA molecule of claim 1, wherein any pyrimidine nucleotides present in the sense strand are 2'-deoxy-2'-fluoro pyrimidine nucleotides and wherein any purine nucleotides present in the sense region are 2'-deoxy purine nucleotides.
16. The siNA molecule of claim 1, wherein the sense strand comprises a 3'-end and a 5'-end, and wherein a terminal cap moiety is present at the 5'-end, the 3'-end, or both of the 5' and 3' ends of said sense strand.
- 20 17. The siNA molecule of claim 16, wherein said terminal cap moiety is an inverted deoxy abasic moiety.
18. The siNA molecule of claim 1, wherein the antisense strand comprises one or more 2'-deoxy-2'-fluoro pyrimidine nucleotides and one or more 2'-O-methyl purine nucleotides.
- 25 19. The siNA molecule of claim 1, wherein any pyrimidine nucleotides present in the antisense strand are 2'-deoxy-2'-fluoro pyrimidine nucleotides and wherein any purine nucleotides present in the antisense strand are 2'-O-methyl purine nucleotides.
20. The siNA molecule of claim 1, wherein the antisense strand comprises a phosphorothioate internucleotide linkage at the 3' end of said antisense strand.
- 30 21. The siNA molecule of claim 1, wherein the antisense strand comprises a glyceryl modification at the 3' end of said antisense strand.

22. The siNA molecule of claim 1, wherein each strand of the siNA molecule comprises 21 nucleotides.
23. The siNA molecule of claim 22, wherein about 19 nucleotides of each strand of the siNA molecule are base-paired to the complementary nucleotides of the other strand of the siNA molecule and wherein at least two 3' terminal nucleotides of each strand of the siNA molecule are not base-paired to the nucleotides of the other strand of the siNA molecule.
24. The siNA molecule of claim 23, wherein each of the two 3' terminal nucleotides of each fragment of the siNA molecule are 2'-deoxy-pyrimidines.
25. The siNA molecule of claim 24, wherein the 2'-deoxy-pyrimidine is 2'-deoxy-thymidine.
26. The siNA molecule of claim 22, wherein 21 nucleotides of each strand of the siNA molecule are base-paired to the complementary nucleotides of the other strand of the siNA molecule.
27. The siNA molecule of claim 22, wherein about 19 nucleotides of the antisense strand are base-paired to the nucleotide sequence of an HCV RNA or a portion thereof.
28. The siNA molecule of claim 22, wherein 21 nucleotides of the antisense strand are base-paired to the nucleotide sequence of an HCV RNA or a portion thereof.
29. The siNA molecule of claim 1, wherein the 5'-end of the antisense strand optionally includes a phosphate group.
30. The siNA molecule of claim 1, wherein the nucleotide sequence of the antisense strand or a portion thereof is complementary to the nucleotide sequence of the 5'-untranslated region of an HCV RNA or a portion thereof.
31. The siNA molecule of claim 1, wherein the nucleotide sequence of the antisense strand or a portion thereof is complementary to the nucleotide sequence of an HCV RNA or a portion thereof that is present in the RNA of all HCV isolates.
32. A pharmaceutical composition comprising the siNA molecule of claim 1 in an acceptable carrier or diluent.
33. A medicament comprising the siNA molecule of claim 1.
34. An active ingredient comprising the siNA molecule of claim 1.

35. The use of a double-stranded short interfering nucleic acid (siNA) molecule that inhibits replication of a hepatitis C virus (HCV), wherein one of the strands of the double-stranded siNA molecule is an antisense strand which comprises a nucleotide sequence that is complementary to the nucleotide sequence of an HCV RNA or a portion thereof and the other strand is a sense strand which comprises a nucleotide sequence that is complementary to the nucleotide sequence of the antisense strand, and wherein a majority of the pyrimidine nucleotides present in the double-stranded siNA molecule comprises a sugar modification.

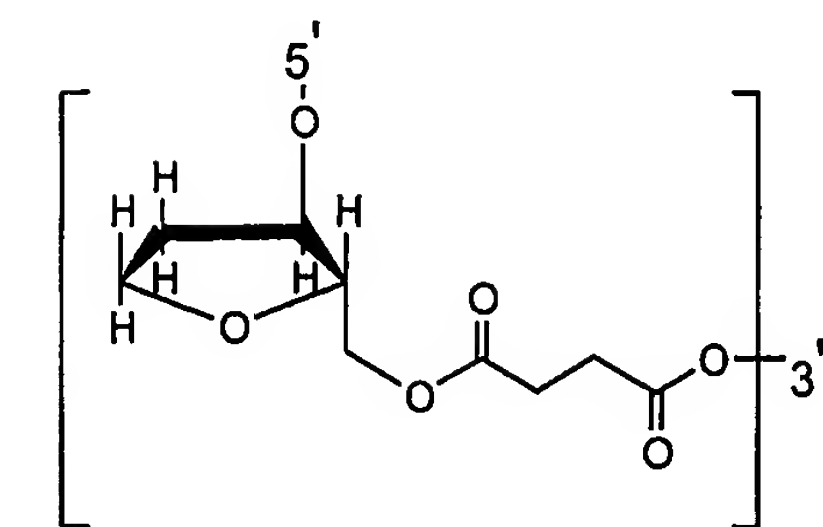
Figure 1

= SOLID SUPPORT

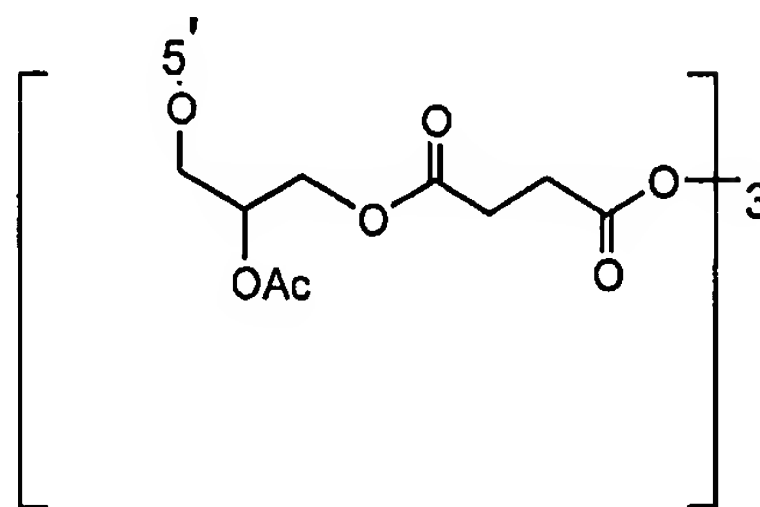
R = TERMINAL PROTECTING GROUP
FOR EXAMPLE:
DIMETHOXYTRITYL (DMT)

(1) = CLEAVABLE LINKER
(FOR EXAMPLE: NUCLEOTIDE SUCCINATE OR
INVERTED DEOXYABASIC SUCCINATE)

(2) = CLEAVABLE LINKER
(FOR EXAMPLE: NUCLEOTIDE SUCCINATE OR
INVERTED DEOXYABASIC SUCCINATE)



INVERTED DEOXYABASIC SUCCINATE
LINKAGE



GLYCERYL SUCCINATE LINKAGE

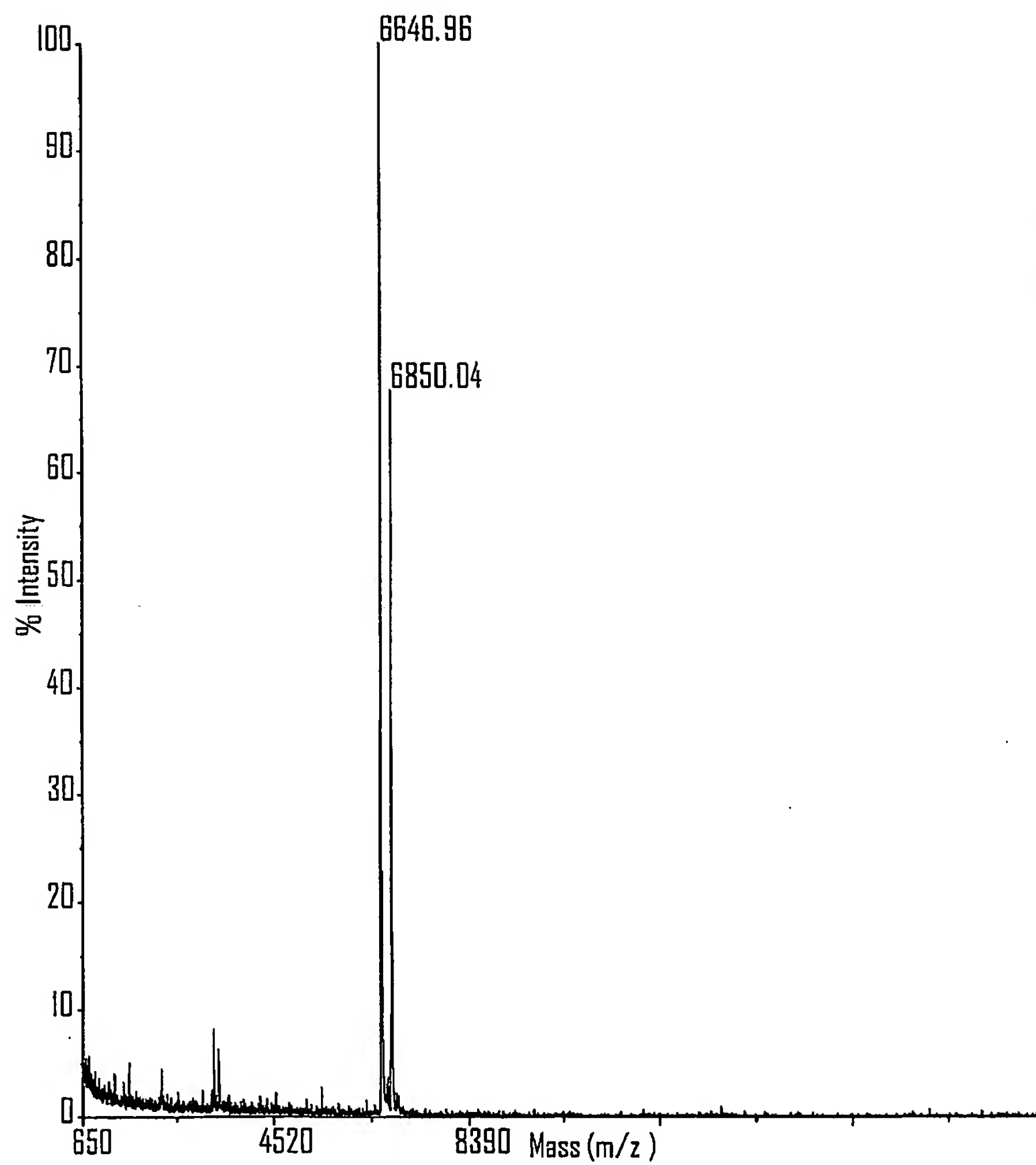
Figure 2

Figure 3

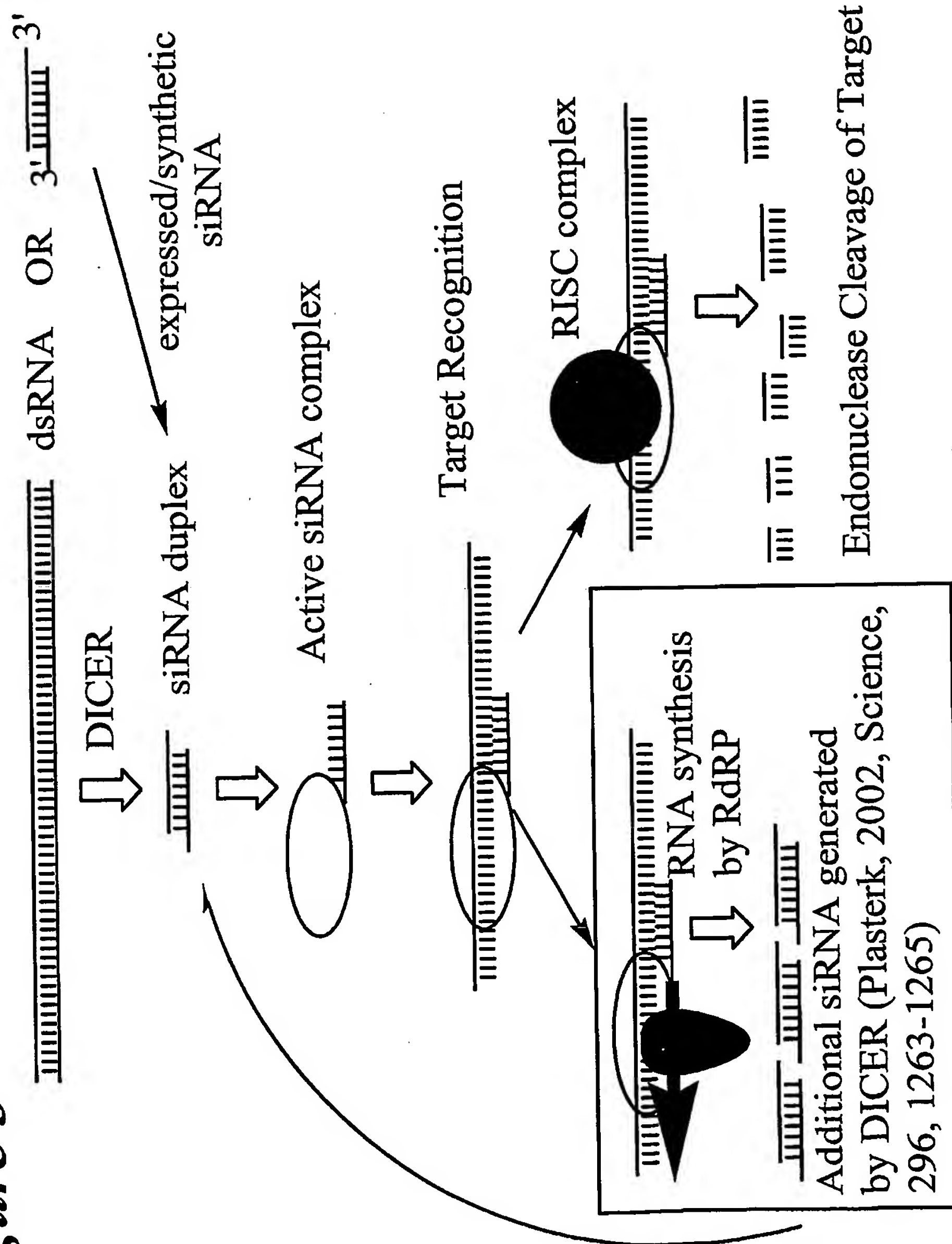
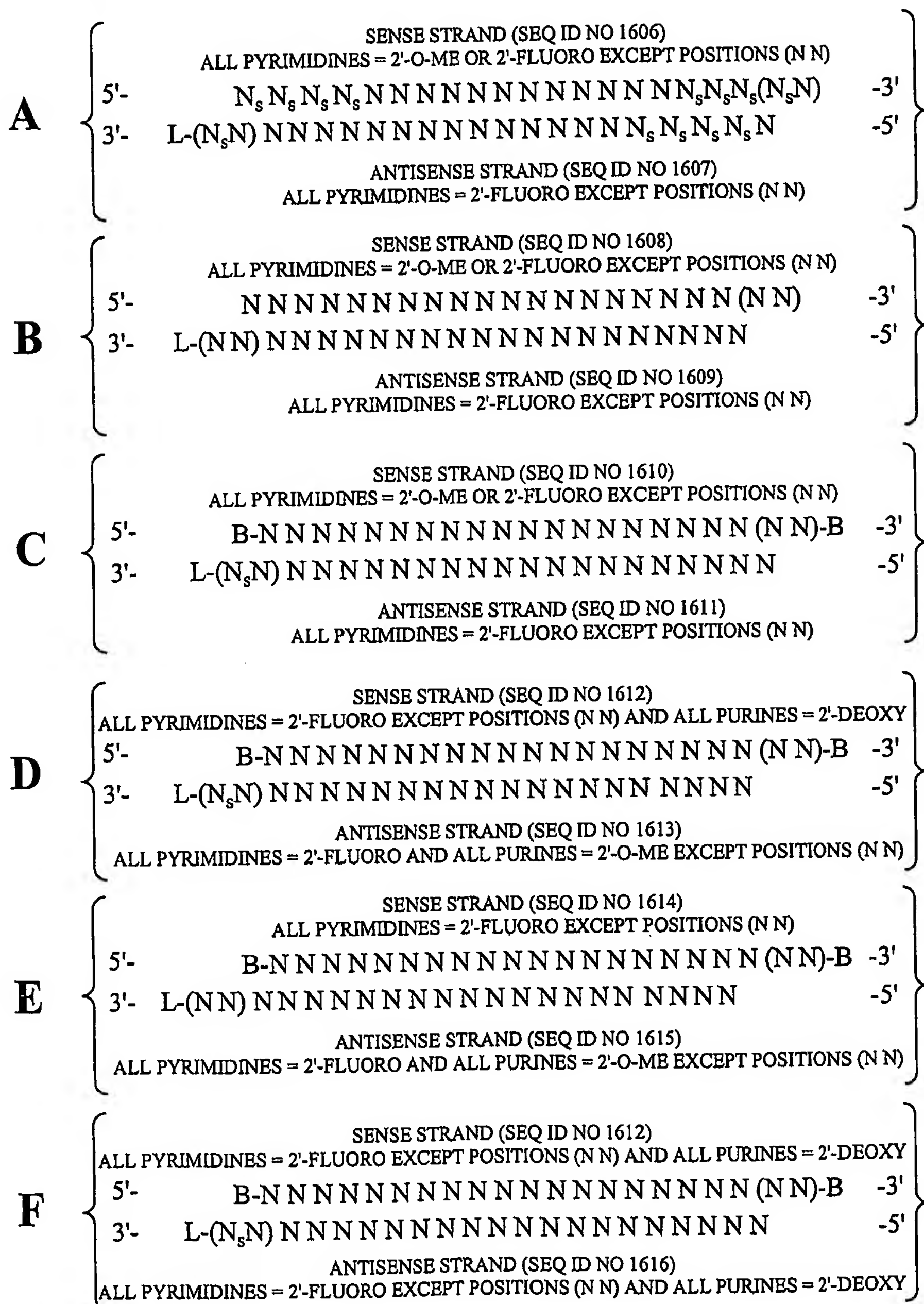


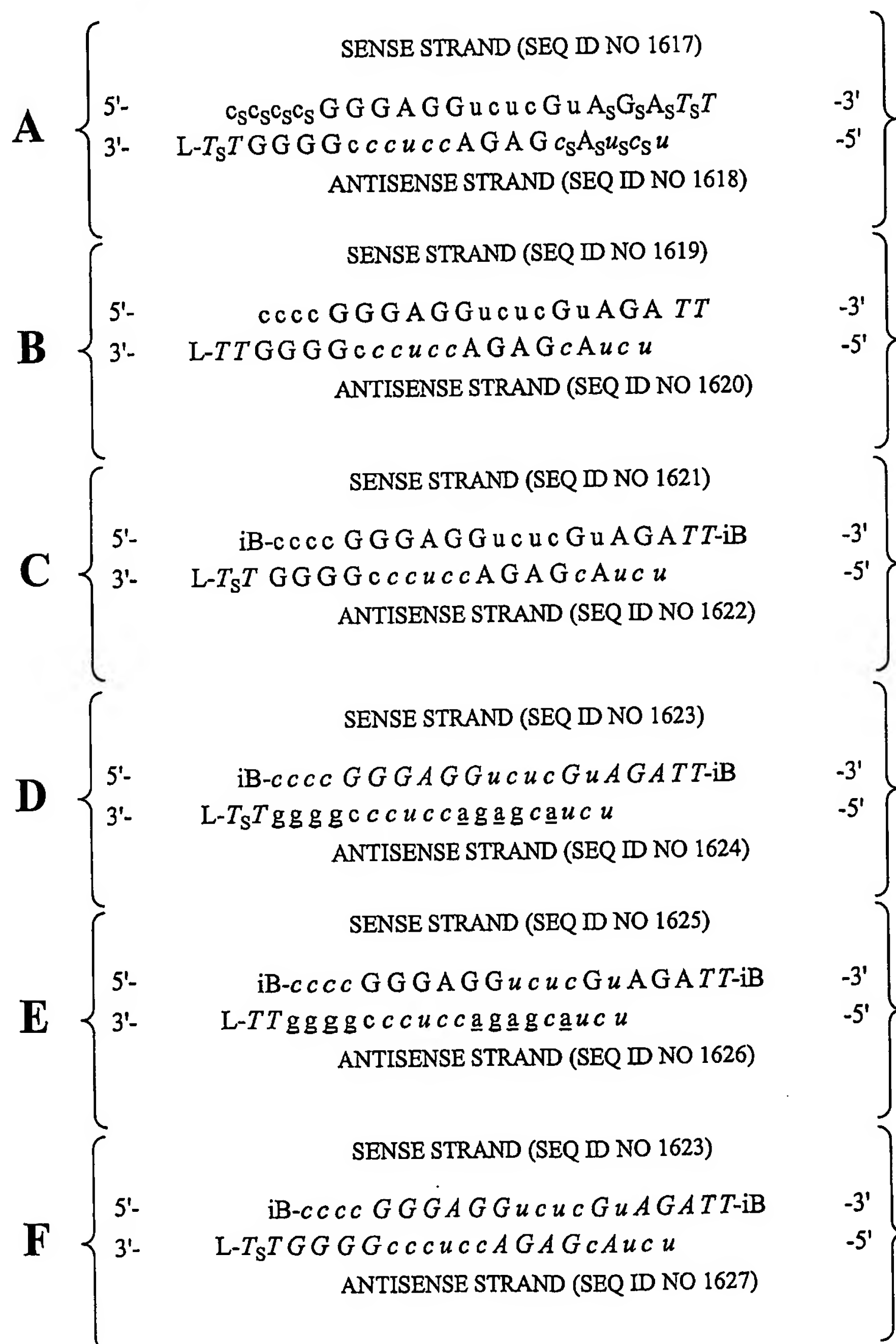
Figure 4

POSITIONS (NN) CAN COMPRISE ANY NUCLEOTIDE, SUCH AS DEOXYNUCLEOTIDES (eg. THYMIDINE) OR UNIVERSAL BASES

B = ABASIC, INVERTED ABASIC, INVERTED NUCLEOTIDE OR OTHER TERMINAL CAP THAT IS OPTIONALLY PRESENT

L = GLYCERYL MOIETY THAT IS OPTIONALLY PRESENT

S = PHOSPHOROTHIOATE OR PHOSPHORODITHIOATE

Figure 5

lower case = 2'-O-Methyl or 2'-deoxy-2'-fluoro
italic lower case = 2'-deoxy-2'-fluoro
underline = 2'-O-methyl

ITALIC UPPER CASE = DEOXY
 B = INVERTED DEOXYABASIC
 L = GLYCERYL MOIETY OPTIONALLY PRESENT
 S = PHOSPHOROTHIOATE OR
 PHOSPHORODITHIOATE

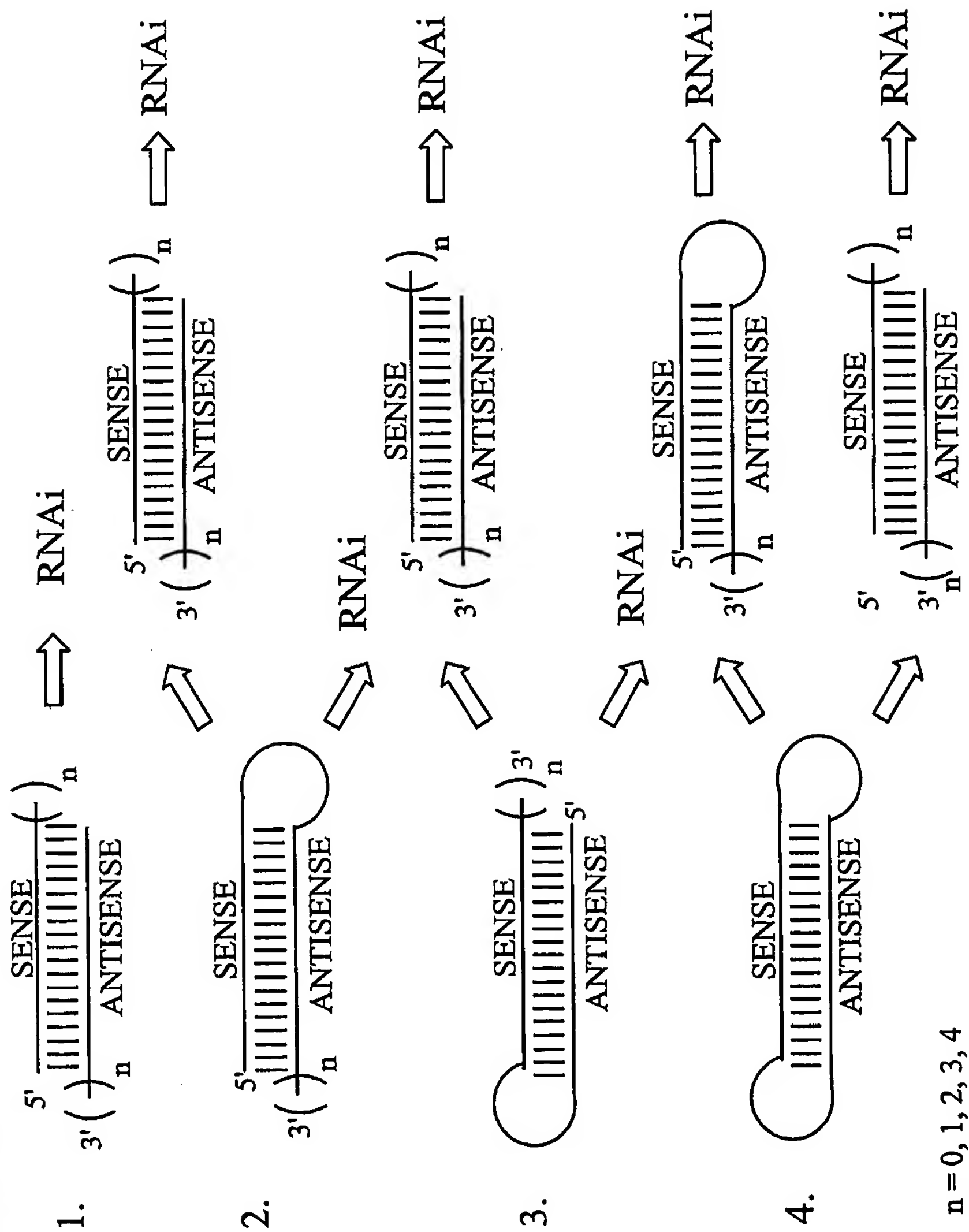
Figure 6

Figure 7

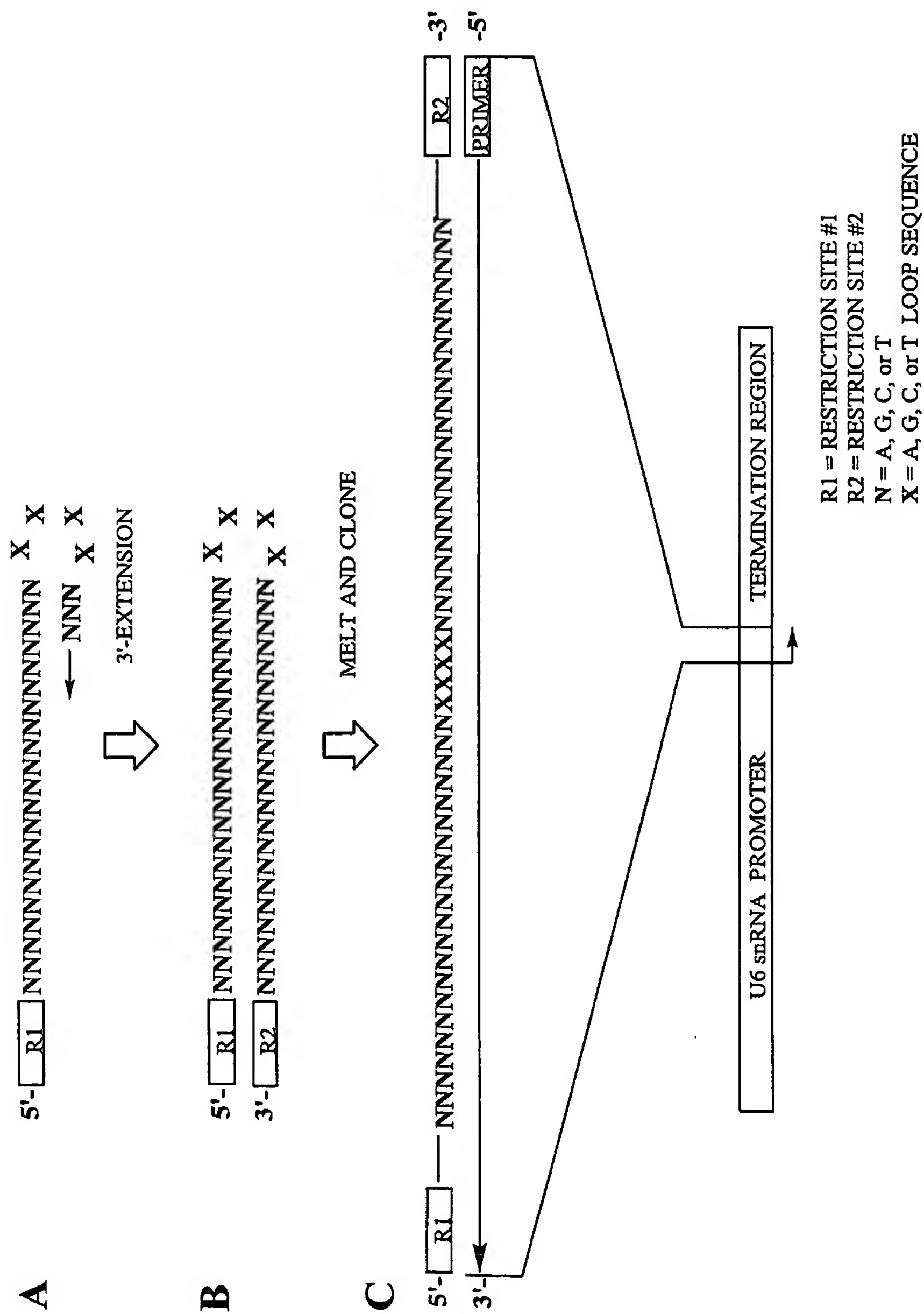


Figure 8

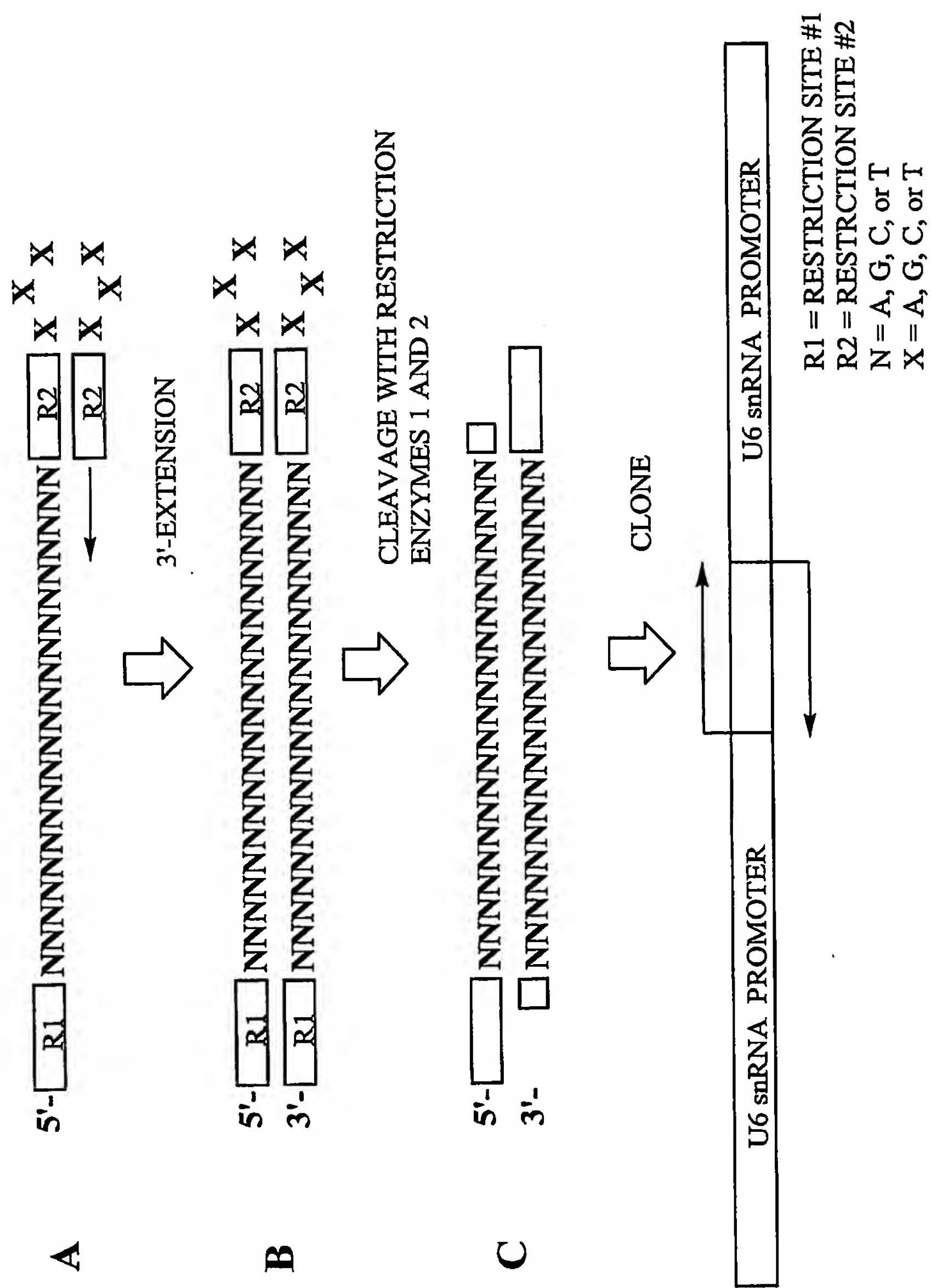


Figure 9: Target site Selection using siRNA

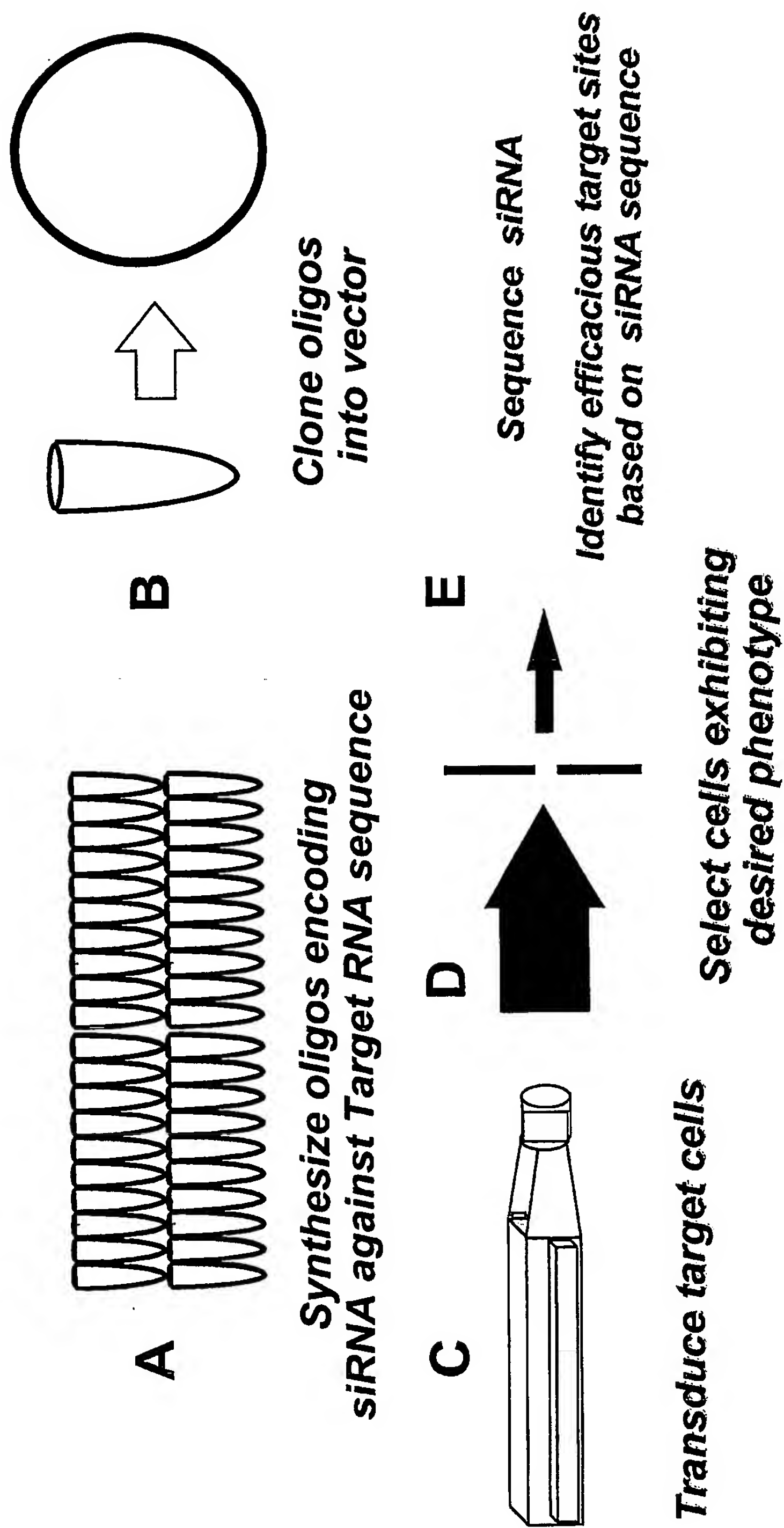
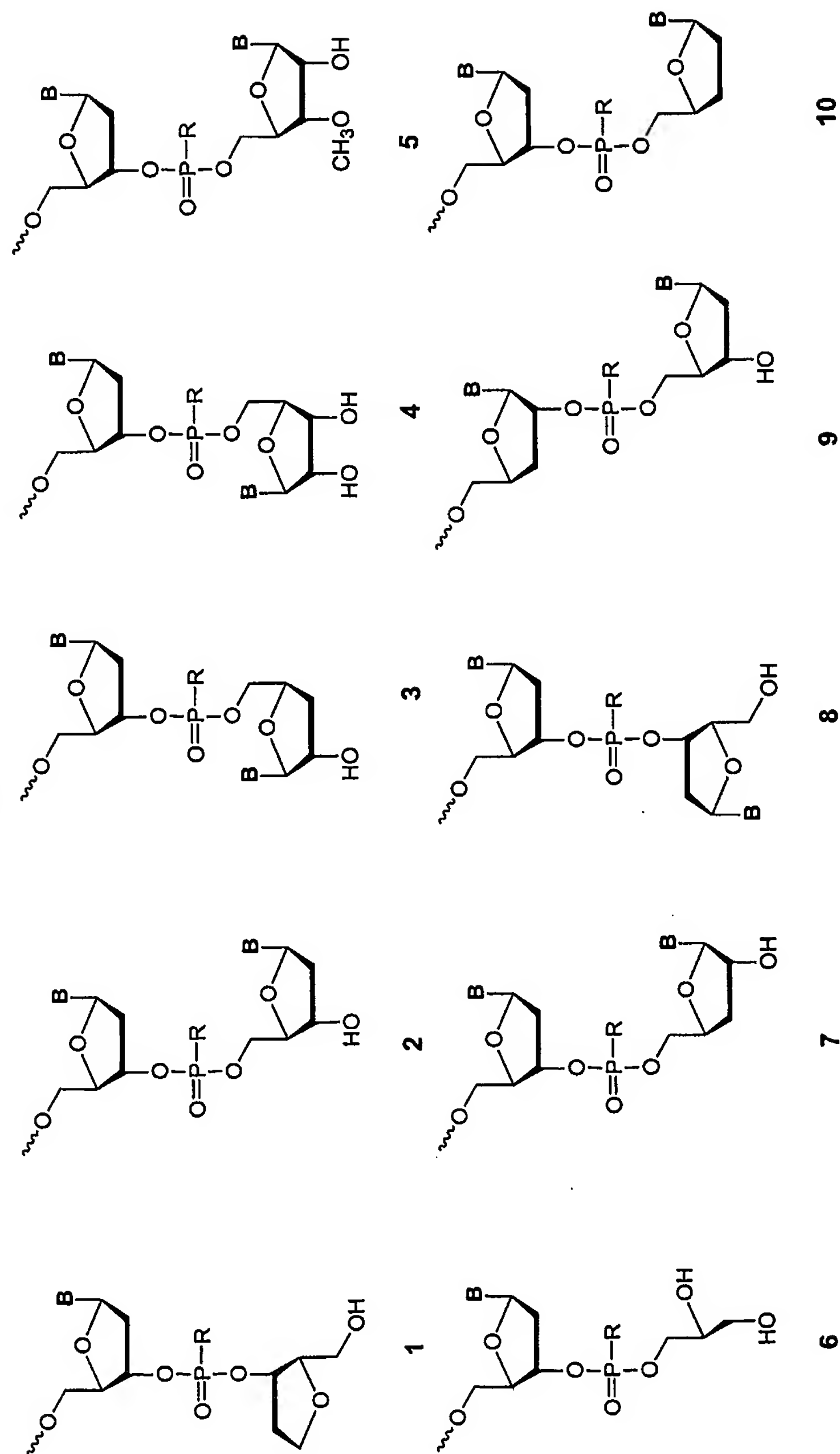


Figure 10

R = O, S, N, alkyl, substituted alkyl, O-alkyl, S-alkyl, alkaryl, or aralkyl
 B = Independently any nucleotide base, either naturally occurring or chemically modified, or optionally H (abasic).

Figure 11: Modification Strategy

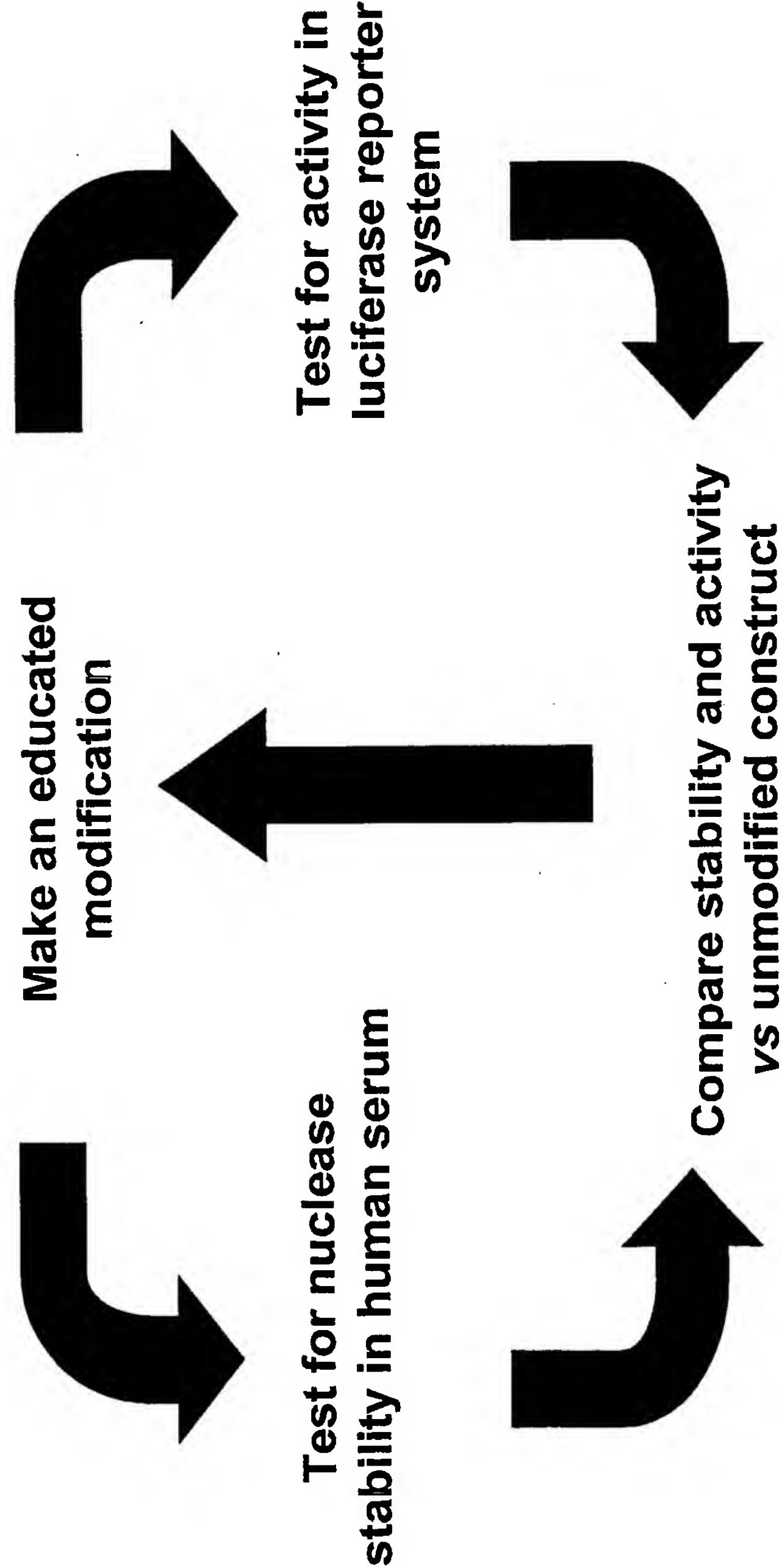


Figure 12 : siRNAs targeting HCV chimera

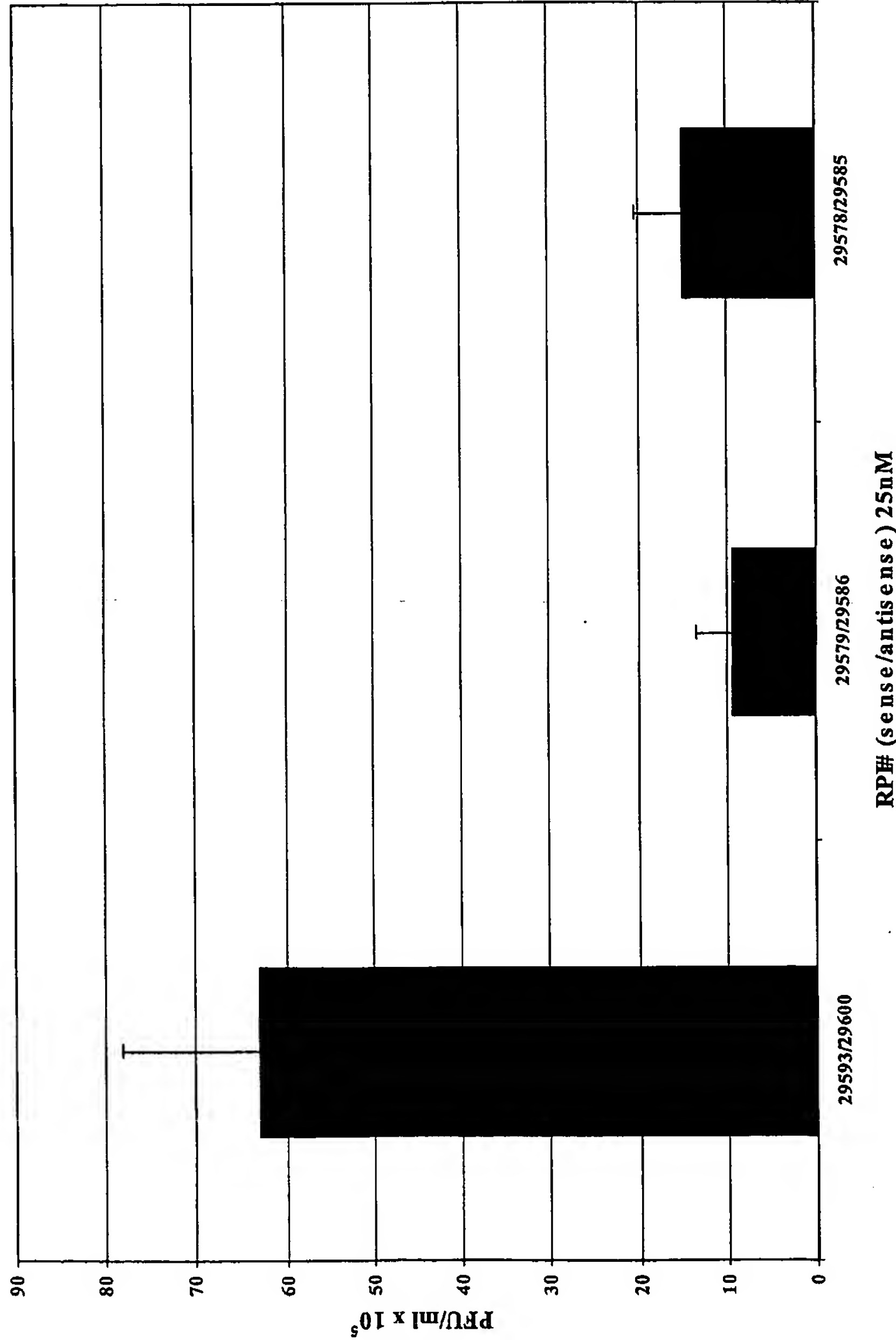
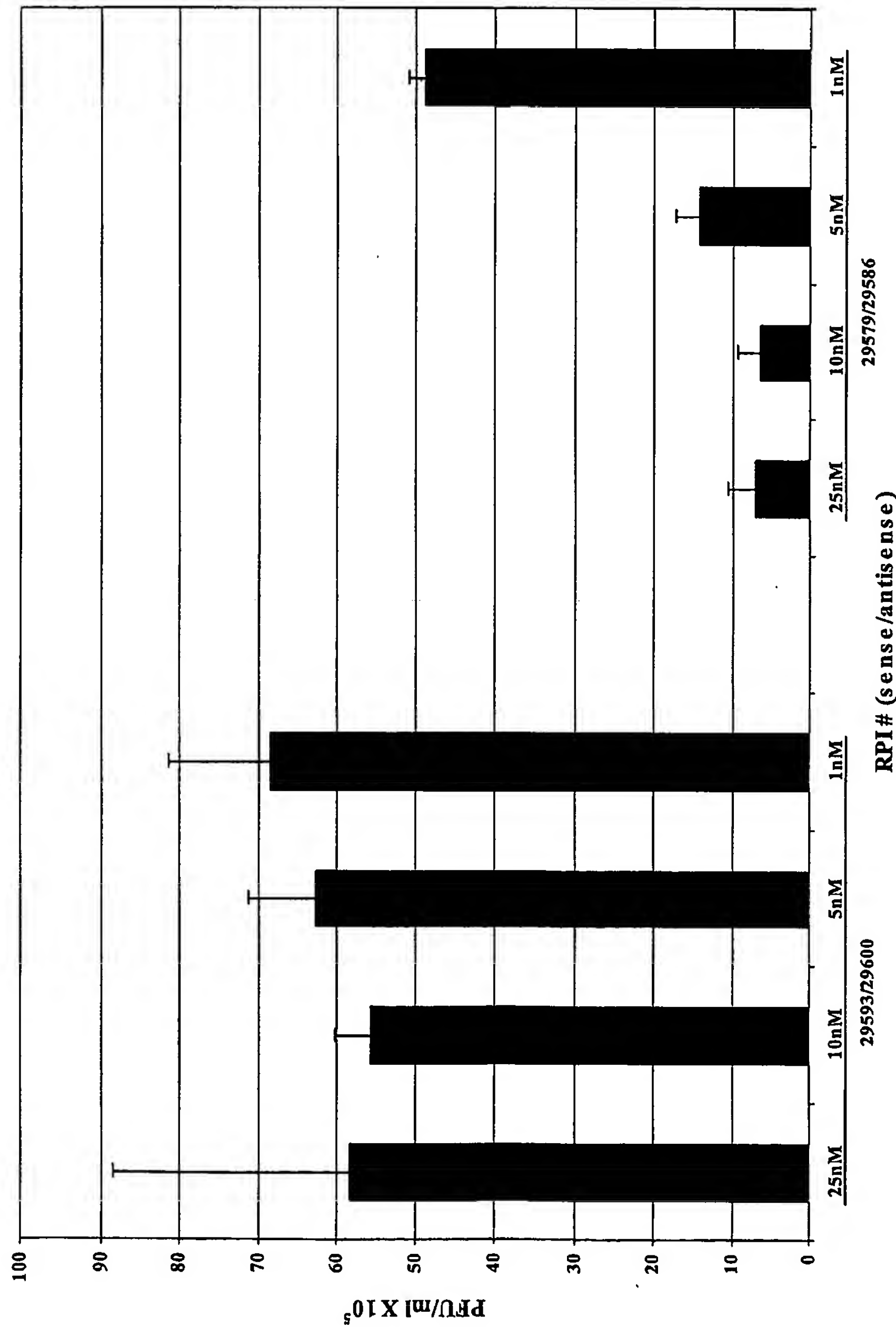
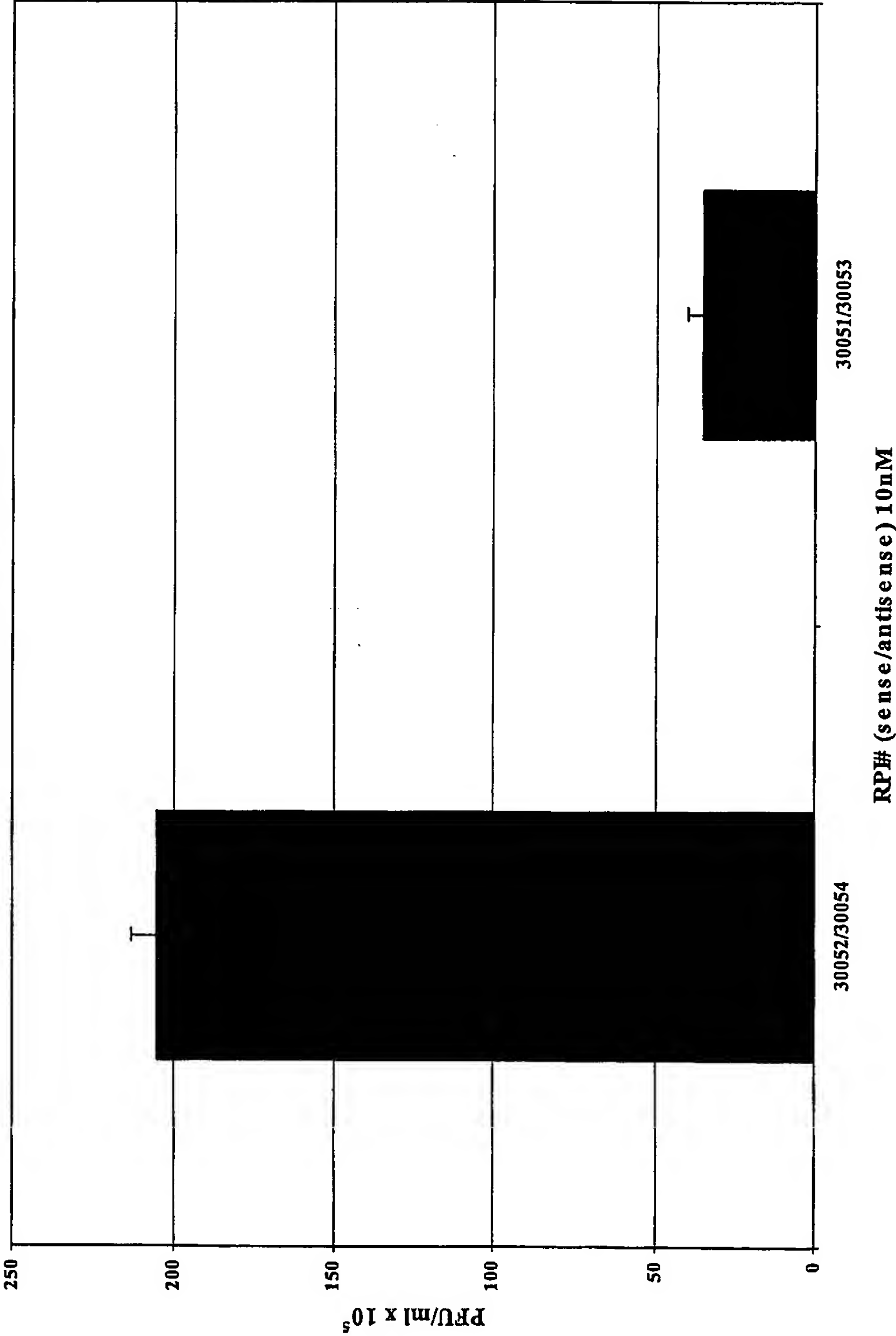


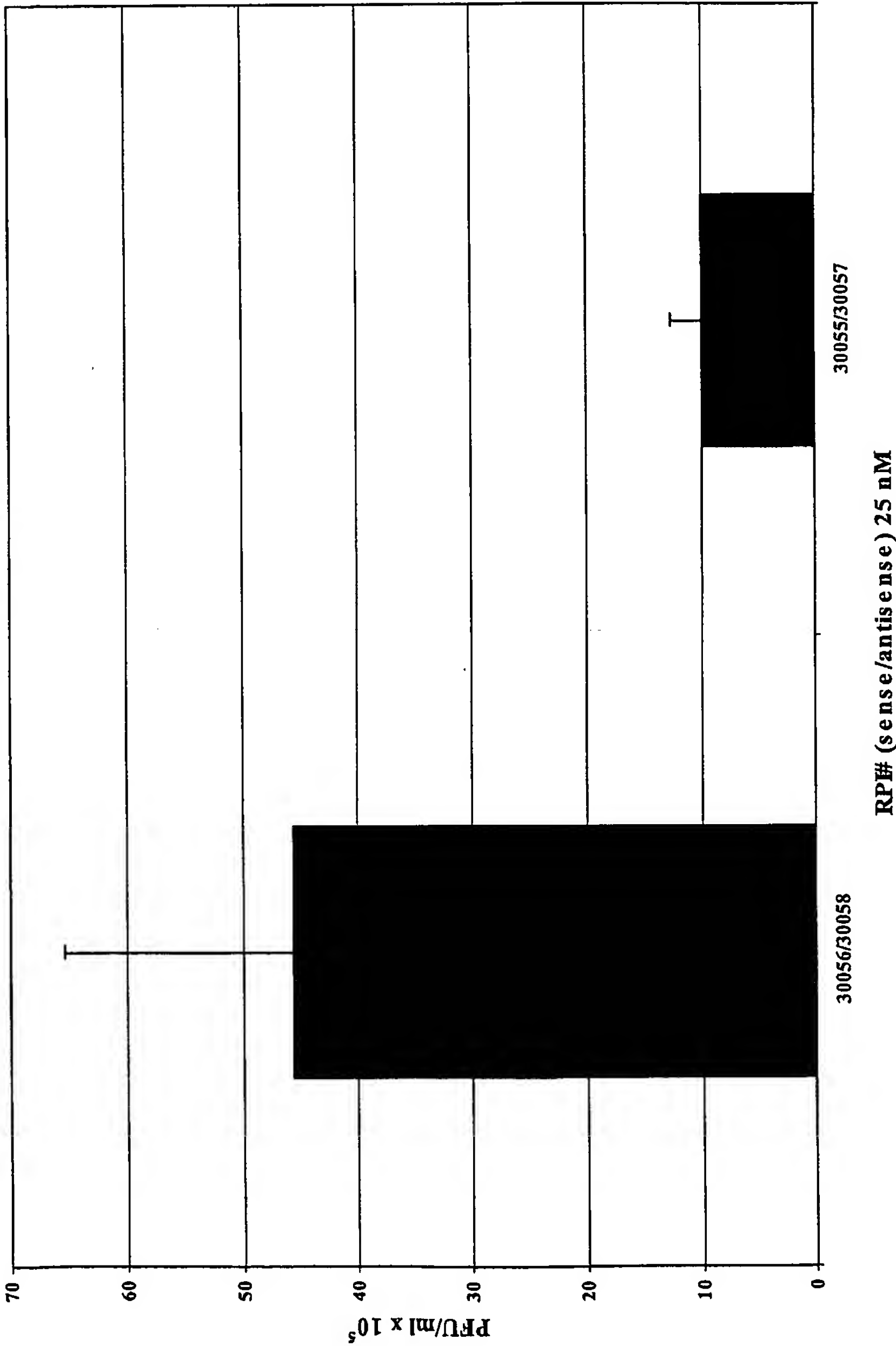
Figure 13: HCV siRNA dose response



*Figure 14: Chemically Modified siRNA targeting
HCV chimera*

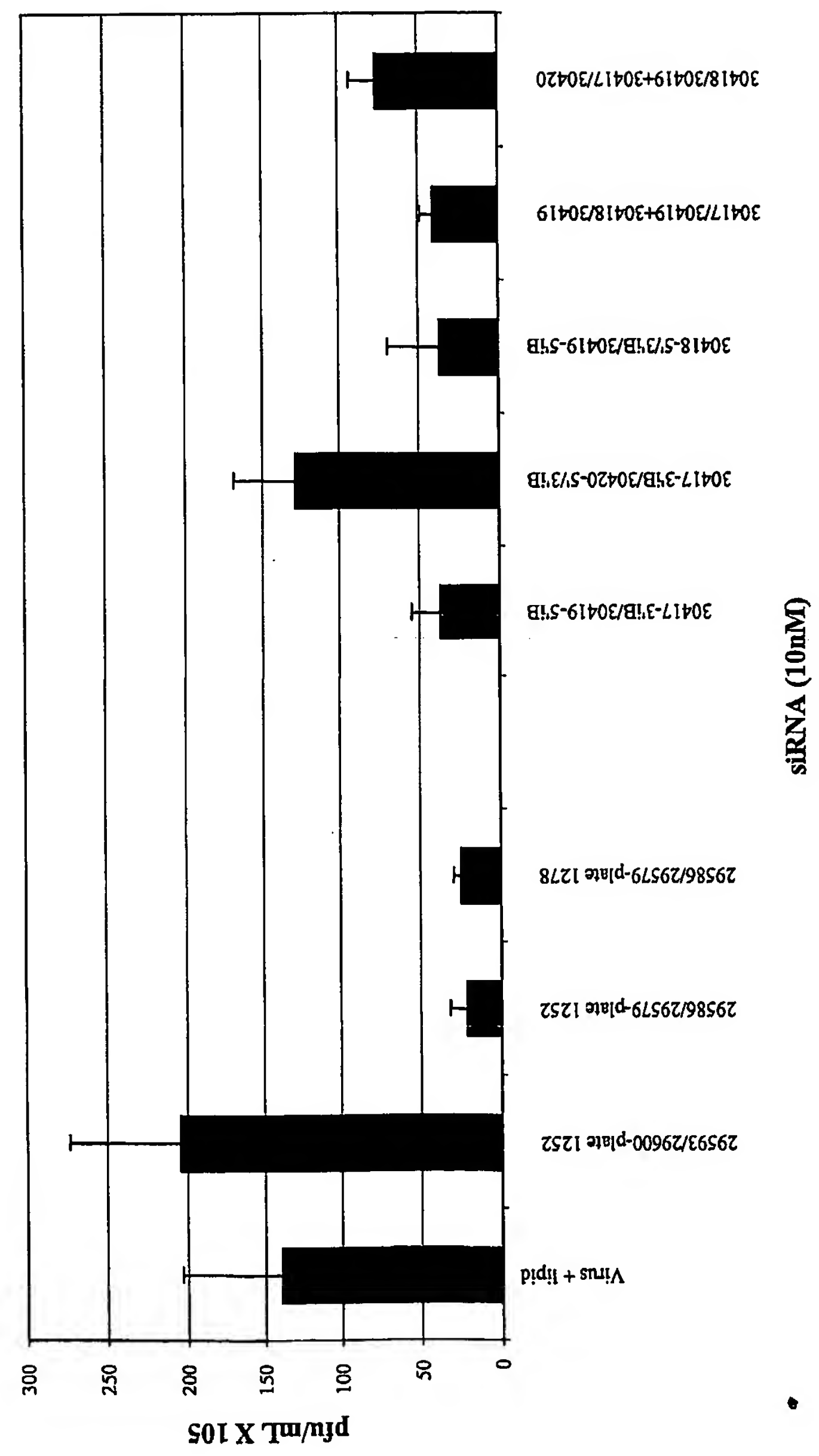


*Figure 15: Chemically Modified siRNA targeting
HCV chimera*



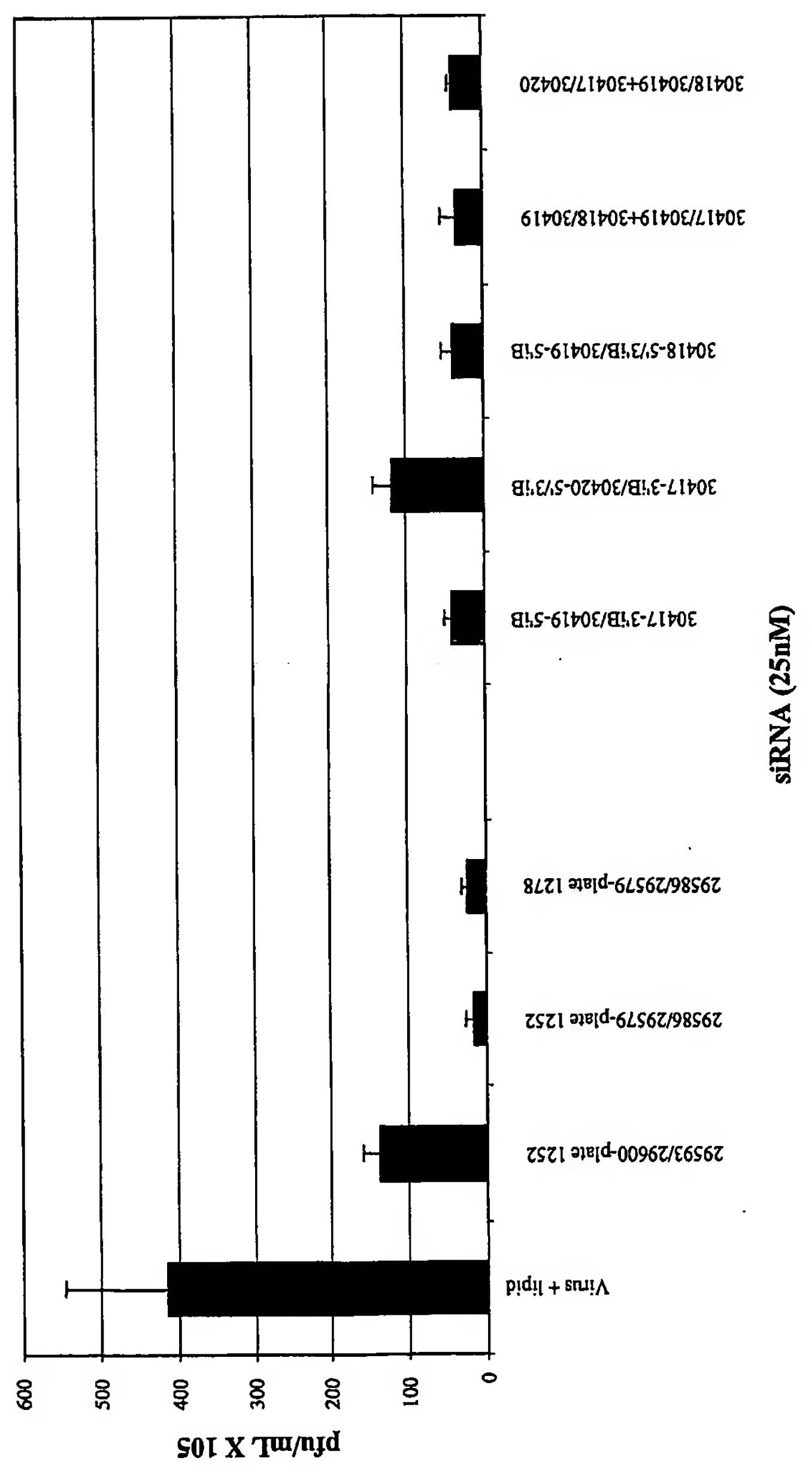
**Figure 16: Chemically Modified siRNA targeting
HCV chimera**

HCV/PV#280-siRNA to HCV-Luc 325/345

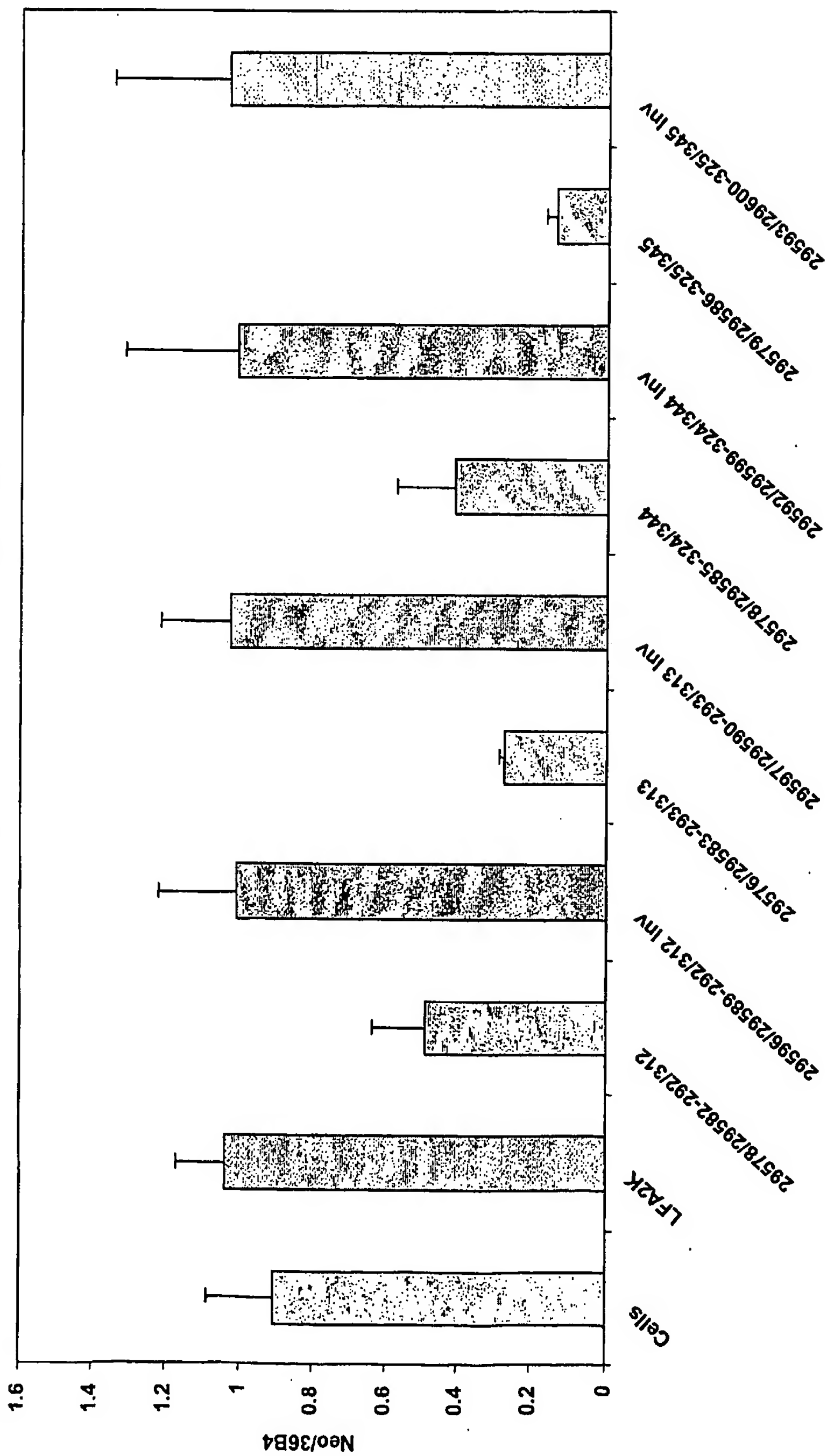


**Figure 17: Chemically Modified siRNA
targeting HCV chimera**

HCV/PV#280-siRNA to HCV-Luc site 325/345



*Figure 18: HCV/Replicon KJ#1-Clone A Cells transfected
with 0.5µl/well LFA 2K-72 hours*



(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property
Organization
International Bureau



(43) International Publication Date
28 August 2003 (28.08.2003)

PCT

(10) International Publication Number
WO 2003/070750 A3

- (51) International Patent Classification⁷: C12Q 1/68, (74) Agent: TERPSTRA, Anita, J.; McDonnell Boehnen Hulbert & Berghoff, Suite 3200, 300 South Wacker Drive, Chicago, IL 60606 (US).
C07H 21/02, 21/04
- (21) International Application Number: PCT/US2003/005043 (81) Designated States (*national*): AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW.
- (22) International Filing Date: 20 February 2003 (20.02.2003)
- (25) Filing Language: English
- (26) Publication Language: English
- (30) Priority Data:
60/358,580 20 February 2002 (20.02.2002) US
60/363,124 11 March 2002 (11.03.2002) US
PCT/US02/09187 26 March 2002 (26.03.2002) US
60/386,782 6 June 2002 (06.06.2002) US
60/401,104 5 August 2002 (05.08.2002) US
60/406,784 29 August 2002 (29.08.2002) US
60/408,378 5 September 2002 (05.09.2002) US
60/409,293 9 September 2002 (09.09.2002) US
60/440,129 15 January 2003 (15.01.2003) US
- (84) Designated States (*regional*): ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PT, SE, SI, SK, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).
- Declaration under Rule 4.17:
— of inventorship (Rule 4.17(iv)) for US only
- (71) Applicant (*for all designated States except US*): SIRNA THERAPEUTICS, INC [US/US]; 2950 Wilderness Place, Boulder, CO 80301 (US). Published:
— with international search report
— before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments
- (72) Inventors; and
- (75) Inventors/Applicants (*for US only*): McSWIGGEN, James [US/US]; 4866 Franklin Drive, Boulder, CO 80301 (US). BEIGELMAN, Leonid [US/US]; 5530 Colt Drive, Longmont, CO 80503 (US). MACEJAK, Dennis [US/US]; 6595 Union Street, Arvada, CO 80004 (US). MORRISSEY, David [US/US]; 4769 Tanglewood Trail, Boulder, CO 80301 (US). (88) Date of publication of the international search report: 18 March 2004
- For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(54) Title: RNA INTERFERENCE MEDIATED INHIBITION OF HEPATITIS C VIRUS

(57) Abstract: The present invention concerns methods and reagents useful in modulating hepatitis C virus (HCV) gene expression in a variety of applications, including use in therapeutic, diagnostic, target validation, and genomic discovery applications. Specifically, the invention relates to small nucleic acid molecules, such as short interfering nucleic acid (siNA), short interfering RNA (siRNA), double-stranded RNA (dsRNA), micro-RNA (miRNA), and short hairpin RNA (shRNA) molecules capable of mediating RNA interference (RNAi) against hepatitis C virus (HCV) gene expression and/or activity. The small nucleic acid molecules are useful in the treatment and diagnosis of HCV infection, liver failure, hepatocellular carcinoma, cirrhosis and any other disease or condition that responds to modulation of HCV expression or activity.

WO 2003/070750 A3

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US03/05043

A. CLASSIFICATION OF SUBJECT MATTER

IPC(7) : C12Q 1/68; C07H 21/02, 21/04

US CL : 435/6; 536/23.1, 24.3, 24.31, 24.33, 24.5

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 435/6; 536/23.1, 24.3, 24.31, 24.33, 24.5

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
Please See Continuation Sheet**C. DOCUMENTS CONSIDERED TO BE RELEVANT**

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y, P	MCCAFFREY et al. RNA interference in adult mice. Nature. 04 July 2002, Vol. 418, pages 38-39, see entire document.	1-35
Y, P	RANDALL et al. Clearance of replicating hepatitis C virus replicon RNAs in cell culture by small interfering RNAs. PNAS. January 2003, Vol. 100, No. 1, pages 235-240, especially page 235, materials and methods section.	1-35
A	FIRE et al. Potent and Specific genetic interference by double-stranded RNA in <i>Caenorhabditis elegans</i> . Nature. 19 February 1998, Vol. 391, pages 806-811, especially page 808, Figure 1.	1-35



Further documents are listed in the continuation of Box C.



See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T"

later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X"

document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y"

document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&"

document member of the same patent family

Date of the actual completion of the international search

10 October 2003 (10.10.2003)

Date of mailing of the international search report

16 JAN 2004

Name and mailing address of the ISA/US

Mail Stop PCT, Attn: ISA/US

Commissioner for Patents

P.O. Box 1450

Alexandria, Virginia 22313-1450

Facsimile No. (703)305-3230

Authorized officer

Janet L. Epps-Ford, Ph.D.

Telephone No. 703-308-0196

Form PCT/ISA/210 (second sheet) (July 1998)

BEST AVAILABLE COPY

INTERNATIONAL SEARCH REPORT

PCT/US03/05043

Continuation of B. FIELDS SEARCHED Item 3:

CAplus, Biosis, Medline, USParfull, Pctfull, EPO, JPO, Derwent

search terms: hepatitis C virus, HCV, dsRNA, short interfering nucleic acid, siRNA, siNA